JOURNAL OF AGRICULTURAL RESEARCH

VOL. X WASHINGTON, D. C., AUGUST 6, 1917 NO. 6

CHEMICAL STUDIES IN MAKING ALFALFA SILAGE

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INTRODUCTION

This paper is a preliminary report on chemical studies in making alfalfa silage. The complete report will appear as a publication of the Kansas Experiment Station. There were two phases of these experiments: One phase was conducted by the Department of Chemistry with quart milk bottles as containers for the silage. The other phase was conducted with seven 10-ton experimental silos in cooperation with the Departments of Bacteriology and Dairy Husbandry,[2] the latter of which built and owns the experimental silos. The first phase of the work was begun in 1912 and continued for four years. The second phase of the work was begun in 1914 and was continued in 1915. In this paper will be given briefly the work of the first three years, followed by a more detailed statement of both phases of the work for 1915.

PART I.—EXPERIMENTS WITH ALFALFA SILAGE MADE IN QUART MILK BOTTLES

PRELIMINARY TRIALS IN 1912 AND 1913

In 1912 only a few preliminary trials were made, but they showed the possibility of success in making silage from alfalfa if the right conditions are discovered. In 1913, 23 bottles were filled, some with alfalfa alone and some with alfalfa plus accessory materials. These materials were corn chop and rye, of which different proportions were used. Some of the alfalfa was merely put through the feed cutter, which made a somewhat finer material than is obtained from the ordinary

[1] Other men in the Department of Chemistry who have been associated with this work are: J. W. Calvin, formerly Assistant Chemist, but now with the University of Nebraska; J. C. Summers, formerly Assistant Chemist, but now with the Operative Miller; J. H. Young, formerly a graduate student, now with Ohio State University, and F. A. Gougler, formerly a graduate student, now County Agent, Johnson County, Missouri.

[2] The authors wish to express their appreciation of the helpful cooperation from Professors O. E. Reed and J. B. Fitch, of the Department of Dairy Husbandry, and Professors L. D. Bushnell and O.W. Hunter, of the Department of Bacteriology. Acknowledgments are also due President H. J. Waters and Dean J. T. Willard for valuable suggestions.

silage cutter. Some of the alfalfa was ground in a large power sausage mill after first being put through the feed cutter. Water was added to these bottles to test the influence of more moisture. The prepared material was compressed in the milk bottles by means of a broom handle, which made a fairly tight packing. Each bottle contained about 1½ pounds of material. No attempt was made in this trial to get exactly the same amount in each bottle. After packing, the mouth was closed with an ordinary cork, which was made fast by wiring with copper wire and sealed with wax such as is used in sealing desiccators.[1] In most of the bottles, as soon as fermentation began, the cork would be pushed out sufficiently to allow the escape of the accumulated gas. The bottles had to be rewired several times. After about two weeks the bottles would stay wired and sealed. These bottles were filled on May 2, 1913, and were opened on January 2, 1914.

QUALITY OF SILAGE PRODUCED

When the bottles were opened, the materials were examined by several persons. Of the 23 bottles, 5 contained silage that was good, and 2 very good. The silage in these two consisted of alfalfa alone ground very fine, the only difference between the two being that the alfalfa in one contained more moisture than that in the other. All of the bottles which contained corn as a part of the mixture graded good. Eight of the bottles graded fair, five bad, and only one very bad. Seven of those that graded fair contained rye as a part of the mixture. But for the strong odor of the rye this silage would have graded good. Rye alone, alfalfa alone cut in the feed cutter, rye and alfalfa with the addition of water, and alfalfa alone plus water all made bad or very bad silage. The addition of water was harmful in all cases this year. In later trials this was not the case. After examination and sampling for analysis, portions of the different kinds were offered to dairy cows. The good and very good were readily eaten. That which graded fair was partially or indifferently eaten.

MOISTURE AND ACIDITY

Five of the bottles which had bad and very bad silage had a moisture content above 75 per cent, while the only bottles of bad silage with a low moisture content were those containing rye alone. A too high or too low water content did not produce good silage. All but one of the bad and very bad bottles of silage had a low percentage of acidity, the only exception being the mixture of alfalfa, rye, and water. All the bottles of silage which graded good had a high percentage of acidity.

[1] This wax is a mixture of 70 parts, by weight, of beeswax, 15 parts of Venice turpentine, and 15 parts of vaseline.

CONCLUSIONS FROM THE WORK OF 1913

The results of the 1913 work show that alfalfa alone makes good silage when finely ground and tightly packed. These conditions are such, however, that it is impossible to realize them in practice. Rye in combination with alfalfa made a fair silage, except for the odor of the rye. Alfalfa alone coarsely ground and loosely packed did not make good silage. Corn chop added to the alfalfa made conditions suitable for good silage to such an extent that there is a possibility of practical realization. The addition of water to green alfalfa was in all cases harmful.

TRIALS TO DETERMINE THE INFLUENCE OF MATURITY

Whether the stage of maturity enters into the conditions for making silage from alfalfa was investigated in the summer of 1914. Alfalfa was cut at four stages of maturity—in the bud, in one-tenth bloom, in full bloom, and in seed—12 bottles of each stage being put up. The alfalfa was all cut in the feed cutter. The 12 conditions were as follows: (1) green alfalfa tightly packed; (2) green alfalfa loosely packed; (3) wilted alfalfa tightly packed; (4) wilted alfalfa tightly packed plus water; (5) corn meal from sound corn and alfalfa in the proportion of 1 to 10; (6) corn meal from germinated corn and alfalfa, 1 to 10; (7) corn meal from germinated corn and alfalfa, 1 to 20; (8) molasses [1] and alfalfa, 1 to 10; (9) molasses and alfalfa, 1 to 20; (10) molasses and alfalfa, 1 to 30; (11) acetic acid and alfalfa, 1 to 50; and (12) lactic acid and alfalfa, 1 to 50. Conditions under 5, 6, and 7 would answer the question whether the more easily fermentable carbohydrates in germinated corn would be more effective than the carbohydrates of sound corn, as well as to show how small an amount of carbohydrate was necessary in order to have the conditions which would produce the requisite amount of acid.

QUALITY OF SILAGE PRODUCED

The bottles were put up in May and opened the following December, and at that time the quality of silage was judged by several persons, as in the previous year. When supplements were added to the alfalfa, the results were 100 per cent good or very good. Molasses as a supplement produced a silage of milder and sweeter odor than did corn meal. The two acids used gave a silage with a sharp acid odor. Corn meal gave an odor that was rather strongly acid. The germinated corn meal gave a sweeter odor, somewhat similar to the molasses. The percentage of good silage when alfalfa was used alone was only 25 for the last three stages, and less than that for the bud stage. Observations made at the time that the bottles were opened were to the effect that the quality of the silage from the bud stage was inferior to that of the three other stages. This experiment showed that alfalfa is suitable for making silage when it is best for hay making.

[1] The use of molasses was suggested to the authors by President Waters.

MOISTURE AND ACIDITY

The figures obtained in the moisture determinations showed that the amount of moisture present may be a favorable condition for the development of the proper amount of acidity, but it is not by itself a determining factor. The general average showed that the bud stage had the highest amount of moisture, that the tenth-bloom and the full-bloom stages were practically equal, and that the seed stage had the lowest amount. These moisture percentages are consistent with the changes in the alfalfa as it matures.

The general average percentage for acidity showed that a high moisture content or a low-moisture content did not necessarily correspond to a proportional development of acidity. The acidity figures also showed that alfalfa alone gave the lowest percentage, and that alfalfa and corn gave the highest percentage.

CONCLUSIONS FROM THE WORK OF 1914

The experiments showed that alfalfa was suitable for making silage when mature enough for making hay; that it was possible to make silage from alfalfa alone when coarsely cut and tightly packed, but in such conditions success was obtained only once in four cases; that if substances containing fermentable carbohydrates were added, the chances of success were 100 per cent.

EXPERIMENTS TO DETERMINE INFLUENCE OF WILTING, TIGHTNESS OF PACKING, ADDITION OF WATER, AND ADDITION OF VARIOUS SUPPLEMENTS

In the spring of 1915, 132 bottles [1] were filled under as many conditions. There were four degrees of tightness of packing, making four bottles in each set. The first bottle was packed as full as possible, and the weight of alfalfa in each was determined. Into the second bottle was put 75 per cent, into the third 50 per cent, and into the fourth 25 per cent as much as into the first bottle. These four bottles will be referred to as first, second, third, and fourth. The supplementary material was mixed with the alfalfa before packing, and as nearly as practicable the relative amount of material was kept uniform. While the material in the first bottle was very tightly packed, more so than is possible in the silo, the second corresponded to tight packing in the silo, and the third to rather loose packing. The alfalfa in the fourth bottle was much more loosely packed than would occur in the ordinary silo.

Alfalfa was used in three conditions; green, wilted, and wilted plus water. All alfalfa was cut in about one-tenth bloom. The wilting was so timed that about one-fourth of the moisture had evaporated. Only an

[1] The complete report will show that 48 additional bottles with air valves were put up, but results from these are omitted entirely from this paper.

approximation in this respect was practicable. Alfalfa in these three conditions was then put into the bottles without supplement, and with supplements consisting of ground sound corn, ground germinated corn, molasses, and rye. The corn was allowed to germinate until the sprouts were about ¼ inch long. The amount of water added to the alfalfa was calculated to be about one-half of that which had evaporated in wilting. The proportions of the various supplements used are given in Table II.

QUALITY OF SILAGE PRODUCED AND EFFECT OF VARIOUS SUPPLEMENTS

On the basis of considering 80 per cent or above good silage, Table I was prepared. From Table II it can be seen that most of those that were good graded 100, only a few being 80 or 90. These figures denote only a shade of deterioration.

TABLE I.—*Distribution of good and poor alfalfa silage in bottles*

Kind of alfalfa silage.	Total number.	Number good.	Number poor.	Per centage good.
Alfalfa alone:				
Fresh	44	30	14	68.2
Wilted	44	42	2	95.4
Wilted+water	44	41	3	93.0
Alfalfa+corn 10:1:				
Total	12	12	0	100.0
Fresh	4	4	0	100.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa+germinated corn 20:1:				
Total	12	12	0	100.0
Fresh	4	4	0	100.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa+germinated corn 40:1:				
Total	12	8	4	66.6
Fresh	4	0	4	0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa+molasses 30:1:				
Total	12	8	4	66.6
Fresh	4	0	4	0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa+rye 3:1:				
Total	12	12	0	100.0
Fresh	4	4	0	100.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa+no supplement:				
Total	12	11	1	91.7
Fresh	4	3	1	75.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
Alfalfa alone:				
Full pack	33	30	3	90.9
75 per cent pack	33	30	3	90.9
50 per cent pack	33	28	5	84.8
25 per cent pack	33	25	8	75.7
With corn 20:1:				
Total	12	9	3	75.0
Fresh	4	4	0	100.0
Wilted	4	2	2	50.0
Wilted+water	4	3	1	75.0
With germinated corn 30:1:				
Total	12	12	0	100.0
Fresh	4	4	0	100.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
With molasses 20:1:				
Total	12	12	0	100.0
Fresh	4	4	0	100.0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
With molasses 40:1:				
Total	12	8	4	66.6
Fresh	4	0	4	0
Wilted	4	4	0	100.0
Wilted+water	4	4	0	100.0
With rye 6:1:				
Total	12	9	3	75.0
Fresh	4	3	1	75.0
Wilted	4	4	0	100.0
Wilted+water	4	2	2	50.0

From a detailed study of the figures in Table I the following conclusions can be drawn:

INFLUENCE OF WILTING.—When fresh alfalfa was used, the percentage of bottles which had good silage was 68.2; for wilted alfalfa the percentage was 95.4, and for wilted alfalfa plus water it was 93.0.

INFLUENCE OF TIGHTNESS OF PACKING.—With a full and a three-fourths pack the percentage of bottles which had good silage was 90.9, with half pack it was 84.8, and with one-fourth pack it was 75.7. The tightness of packing is an advantage, in that it makes exclusion of air and retention of carbon dioxid more certain. A detailed study of the chemical data obtained showed that favorable development of acid took place if air was kept out.

INFLUENCE OF SOUND CORN AS A SUPPLEMENT.—When the proportion of sound corn added to the alfalfa was 1 to 10, the percentage of bottles having good silage was 100 for the fresh, for the wilted, and for the wilted plus water. When the proportion of sound corn to alfalfa was 1 to 20, the percentage of all bottles having good silage was 75. When fresh alfalfa was used, the percentage was 100; with wilted alfalfa it was 50; and with wilted alfalfa plus water it was 75. The results from the proportion of 1 to 20 are so contradictory that no conclusions can be drawn. Sound corn in the proportion of 1 to 10 is very effective if air is kept out.

INFLUENCE OF GERMINATED CORN AS A SUPPLEMENT.—With the proportion of 1 to 20 the percentage of all bottles having good silage was in all cases 100 for the fresh, for the wilted, and for the wilted plus water. The same results were obtained with the proportion of 1 to 30. With the proportion of 1 to 40 the percentage of all bottles having good silage was 66.6. When fresh alfalfa was used, none were good. When wilted alfalfa or wilted alfalfa plus water was used, the percentage of good silage was 100. Germinated corn is more effective as a supplement than sound corn. If too small an amount is used, the chances of success with fresh alfalfa are no better than if none were used. Wilting is an advantage.

INFLUENCE OF MOLASSES AS A SUPPLEMENT.—With the proportion of 1 to 20 the percentage of all bottles having good silage was 100 for the fresh, for the wilted, and for the wilted plus water. With the proportions 1 to 30 and 1 to 40 the percentage of bottles having good silage was in all cases 100, except with the fresh alfalfa, where they were all bad. In general, it may be said that molasses as a supplement has the same effectiveness as germinated corn.

RYE AS A SUPPLEMENT.—With the proportion 1 to 3 the percentage of all bottles having good silage was in all cases 100. With the proportion 1 to 6 the percentage of all bottles having good silage was 75; with fresh alfalfa it was 75, with wilted alfalfa it was 100, and with wilted alfalfa plus water it was 50. In general, it may be said that rye as a supplement was very effective when the alfalfa was wilted. If fresh, the amount of moisture seems to be too large for the formation of good silage.[1]

[1] "Good silage" as used in this paragraph means that putrefactive odors were absent. The rye, however, imparts a strong, peculiar odor of its own, but this is different from the odors due to putrefactive decomposition.

PRIMARY CONDITIONS FOR MAKING ALFALFA SILAGE

This analysis of the figures presented leads to the conclusion that the primary conditions for making good alfalfa silage are exclusion of air and retention of carbon dioxid. The use of a supplement is necessary to insure success because it is not possible in practice to fulfill these conditions absolutely.

CHEMICAL COMPOSITION OF SILAGE FROM BOTTLES

Table II gives the figures for the chemical analysis of the silage put up in the 132 bottles in the spring of 1915. After judging the quality at the time of opening, the silage was analyzed for moisture, acidity, total nitrogen, and nitrogen in amino form as determined by the formol titration. The quality of the silage was expressed on a percentage basis. When the quality of the silage from the four bottles in a set (full, three-fourths, one-half, and one-fourth pack) graded 90 to 100, only the average figures from the analysis of the four bottles are given in the tables. It was found that the tightness of packing made comparatively little difference in the percentage amount of chemical change produced.

TABLE II.—*Chemical analysis of alfalfa silage in bottles*

MADE FROM FRESHLY CUT ALFALFA

Kind of alfalfa silage and bottle No.	Grade.	Moisture.	Acidity for 5 gm.	Amino nitrogen for 5 gm.	Acidity.	Amino nitrogen.	Total nitrogen.	Ratio of amino nitrogen to total nitrogen.
	Per cent.	*Per cent.*	*C. c.*	*C. c.*	*Per cent.*	*Per cent.*	*Per cent.*	
Alfalfa alone:								
1	[a] 100	76.86	19.0	14.0	1.710	0.196	0.641	1:3.26
2	100	75.45	18.0	14.2	1.701	.199	.682	1:3.43
3	100	79.90	17.0	13.9	1.526	.195	.551	1:2.83
4	0	82.80	13.4	17.8	1.206	.249	.306	1:1.23
Average of 1, 2, and 3	100	77.40	18.0	14.0	1.646	.197	.625	1:3.18
Alfalfa + sound corn 10:1, average	100	72.49	27.8	15.6	2.498	.218	.640	1:2.94
Alfalfa + sound corn 20:1, average	100	74.54	25.3	14.7	2.275	.206	.639	1:3.10
Alfalfa + germinated corn 20:1, average	100	74.65	32.5	15.0	2.927	.210	.708	1:3.37
Alfalfa + germinated corn 30:1, average	[b] 100	76.48	29.9	15.9	2.687	.218	.656	1:3.01
Alfalfa + germinated corn 40:1:								
21	50	77.75	15.9	21.9	1.431	.307	.543	1:1.77
22	50	77.85	18.6	22.2	1.674	.291	.508	1:1.75
23	60	80.10	18.2	22.6	1.638	.316	.393	1:1.24
24	40	79.20	17.5	25.0	1.575	.330	.478	1:1.37
Average of 21, 22, 23, 24	.....	78.73	17.6	22.9	1.580	.316	.481	1:1.52
Alfalfa + molasses 20:1, average	100	75.83	26.6	15.3	2.392	.214	.670	1:3.13

[a] In the groups where results are given on each bottle separately, the first one is full pack, the second 75 per cent, the third 50 per cent, and the fourth 25 per cent.
[b] The bottle with 25 per cent pack graded only 90.

TABLE II.—*Chemical analysis of alfalfa silage*—Continued

MADE FROM FRESHLY CUT ALFALFA—continued

Kind of alfalfa silage and bottle No.	Grade.	Moisture.	Acidity for 5 gm.	Amino nitrogen for 5 gm.	Acidity.	Amino nitrogen.	Total nitrogen.	Ratio of amino nitrogen to total nitrogen.
	Per cent.	*Per cent.*	*C. c.*	*C. c.*	*Per cent.*	*Per cent.*	*Per cent.*	
Alfalfa+molasses 30:1:								
29	40	80.40	13.4	22.5	1.202	.315	.398	1:1.27
30	40	79.95	12.6	22.4	1.134	.314	.401	1:1.29
31	50	79.95	13.2	21.3	1.188	.298	.412	1:1.38
32	70	80.00	19.1	17.7	1.719	.248	.554	1:2.23
Average of 29, 30, 31, 32		80.08	14.6	21.0	1.411	.294	.441	1:1.50
Alfalfa+molasses 40:1:								
33	50	78.50	15.0	21.0	1.350	.294	.408	1:1.39
34	50	80.55	16.0	21.6	1.440	.302	.399	1:1.33
35	60	79.90	19.4	20.1	1.746	.281	.411	1:1.46
36	50	81.20	16.1	23.1	1.449	.323	.398	1:1.23
Average of 33, 34, 35, 36		80.04	16.6	21.5	1.496	.300	.404	1:1.35
Alfalfa+rye 3:1, average	100	68.59	27.5	18.3	2.475	.255	.705	1:2.76
Alfalfa+rye 6:1:								
41	100	68.95	27.0	17.4	2.426	.244	.711	1:2.91
42	100	71.20	24.4	17.8	2.196	.249	.689	1:2.76
43	100	71.60	24.1	18.8	2.165	.263	5.23	1:1.99
44	25	74.00	12.5	22.6	1.121	.316	.554	1:1.75
Average of 41, 42, 43		70.58	25.2	18.0	2.262	.252	.641	1:2.54

MADE FROM WILTED ALFALFA

Alfalfa alone, average	[a] 100	68.96	28.0	24.7	2.522	0.368	0.855	1:2.33
Alfalfa+sound corn 10:1, average	100	62.68	34.9	24.2	3.143	.339	.887	1:2.62
Alfalfa+sound corn 20:1:								
57	100	64.05	35.4	25.1	3.186	.383	.992	1:2.59
58	100	62.75	34.8	25.2	3.132	.286	1.028	1:3.60
59	50	68.00	19.5	33.5	1.751	.469	.717	1:1.53
60	25	69.80	14.3	30.0	1.287	.420	.691	1:1.64
Average of 57, 58		63.40	35.1	25.2	3.159	.335	1.010	1:3.02
Alfalfa+germinated corn 20:1, average	100	60.14	35.8	29.5	3.222	.413	1.170	1:2.83
Alfalfa+germinated corn 30:1, average	[a] 100	60.5	36.2	29.5	3.265	.412	1.172	1:2.85
Alfalfa+germinated corn 40:1, average	[a] 100	58.01	35.6	28.7	3.206	.401	1.209	1:3.01
Alfalfa+molasses 20:1, average	[a] 100	61.58	37.4	25.9	3.370	.363	1.115	1:3.08
Alfalfa+molasses 30:1, average	100	60.26	36.9	26.0	3.328	.364	1.190	1:3.27
Alfalfa+molasses 40:1, average	100	60.66	35.0	23.9	3.148	.335	1.126	1:3.37
Alfalfa+rye 3:1, average	100	68.24	31.2	23.1	2.540	.323	.741	1:2.30
Alfalfa+rye 6:1, average	100	66.34	30.3	28.1	2.477	.392	.894	1:2.28

[a] The bottle with 25 per cent pack graded only 90 or 95.

TABLE II.—*Chemical analysis of alfalfa silage*—Continued

MADE FROM WILTED ALFALFA PLUS WATER

Kind of alfalfa silage and bottle No.	Grade.	Moisture.	Acidity for 5 gm.	Amino nitrogen for 5 gm.	Acidity.	Amino nitrogen.	Total nitrogen.	Ratio of amino nitrogen to total nitrogen.
	Per cent.	*Per cent.*	*C. c.*	*C. c.*	*Per cent.*	*Per cent.*	*Per cent.*	
Alfalfa+water, average	100	73.36	25.6	23.5	2.302	.329	.685	1:2.08
Alfalfa+water+sound corn 10:1, average	[a] 100	65.46	34.8	23.5	3.131	.329	.877	1:2.67
Alfalfa+water+sound corn 20:1:								
65	100	69.10	36.4	24.0	3.276	.336	.840	1:2.50
66	95	68.60	34.8	22.9	3.132	.321	.838	1:2.61
67	90	69.70	31.3	24.6	2.817	.344	.800	1:2.33
68	50	72.00	25.7	27.3	2.313	.382	.707	1:1.85
Average of 65, 66, 67	.....	69.13	34.2	23.8	3.075	.334	.826	1:2.47
Alfalfa+water+germinated corn 20:1, average	100	66.65	36.9	22.2	3.328	.312	.920	1:2.95
Alfalfa+water+germinated corn 30:1, average	100	66.74	35.9	24.6	3.242	.331	.962	1:2.91
Alfalfa+water+germinated corn 40:1, average	100	65.80	37.3	28.7	3.227	.401	1.014	1:2.52
Alfalfa+water+molasses 20:1, average	100	65.55	36.1	23.5	3.255	.329	.953	1:2.90
Alfalfa+water+molasses 30:1, average	100	66.41	35.9	23.2	3.244	.324	.903	1:2.79
Alfalfa+water+molasses 40:1, average	100	72.19	34.2	20.7	3.074	.290	.771	1:2.66
Alfalfa+water+rye 3:1, average	100	68.30	30.4	23.2	2.734	.324	.775	1:2.39
Alfalfa+water+rye 6:1:								
129	90	70.40	26.0	25.7	2.340	.360	.778	1:2.16
130	80	69.80	27.5	25.3	2.475	.354	.769	1:2.17
131	70	71.30	26.4	25.5	2.376	.357	.730	1:2.04
132	70	70.40	25.1	24.4	2.255	.342	.758	1:2.21
Average of 129, 130, 131, 132	.....	70.48	26.3	24.2	2.362	.343	.759	1:2.22

[a] The bottle with 25 per cent pack graded only 90.

MOISTURE CONTENT.—The moisture was determined by drying 100 gm. in a steam oven for 10 hours. As it is best to use the sample in its original condition without any previous preparation, a large sample is taken. Preliminary trials showed that 10 hours were a sufficient length of time to bring these samples to a fairly constant weight. It is practically impossible to get a constant weight because of the amount of lactic and acetic acids present. All samples were dried a second time, however, in order to check accidental errors. The bottles containing silage from fresh alfalfa had a higher moisture content than the bottles containing wilted alfalfa and wilted alfalfa plus water. The sets of four where all the bottles had bad silage had a higher moisture content than any other sets. In individual cases bad silage had no more moisture than that which was good, the moisture content being only one factor of several that may cause poor silage; but too high moisture content is undesirable. The difference

in moisture content between the silage from wilted alfalfa and from wilted alfalfa plus water is not as great as would be expected. This is no doubt due to the fact that during the first two weeks' fermentation moisture was lost because the stoppers could not be kept in place.

ACIDITY CONTENT.—Acidity was determined by the method of extracting in 50 per cent alcohol. Investigations made at this laboratory [1] had shown that the method of water extraction did not give as high a percentage of acidity as the extraction with alcohol. The method used was as follows: Two hundred gm. of carefully mixed silage are passed through the clover cutter and are then placed in a 2-quart Mason jar. For well-mixed alfalfa silage where only total acidity is to be determined half of this amount is sufficient. For mixed silage from a silo or for ordinary corn silage it is best to use this size of sample, as there is a sufficiency of extract for other determinations, such as volatile acids and nitrogen in amino form. To the silage in the Mason jar are added 500 c. c. of 95 per cent neutral alcohol. The advantage of this procedure is that when the determination can not be finished at once the alcohol will stop bacterial and enzymic action, and the sample can be kept as long as desired. The amount of water present in the sample, as known from the moisture determination, is calculated, and water equivalent to the difference between this and 500 c. c. is added to the jar, making the total quantity of liquid in contact with the silage 1,000 c. c. The jar is then placed on a shaking machine for about six hours, after which the contents are thrown on a 32 cm. folded filter. Triplicate portions of 25 c. c. of this filtrate are titrated with *N/20* sodium hydroxid, using phenolphthalein as the indicator.

The figures for acidity are probably large. The titration was made on a portion of extract representing 5 gm. This means that the analytical error in computing to percentage is multiplied 20 times. The extract is highly colored, and the end point is not easily read. To compensate for this, all the acidity titrations, as well as the formol titrations, were made by the same person during the last two years of work. This gives the figures greater comparative value.

The results for acidity titration are expressed as cubic centimeters of *N/20* sodium hydroxid for the extract from 5 gm. of silage, and also as percentages of lactic acid. This gives a higher percentage of acidity than the reality; but as only comparative values are needed here, this method of calculation is sufficiently accurate. Farther on attention will be called to the relative amount of volatile and nonvolatile acids in alfalfa silage made with various supplements. The results for acidity, as well as for moisture, total nitrogen, and amino nitrogen, were averaged for each set of four bottles; but only those of approximately equal quality on the basis of percentage grade were included in this average.

[1] SWANSON, C. O., CALVIN, J. W., and HUNGERFORD, Edwin. ACIDITY IN SILAGE; METHOD OF DETERMINATION. *In* Jour. Amer. Chem. Soc., v. 35, no. 4, p. 476-483. 1913.

The results from bottles having a grade above 90 should manifestly not be averaged with those having a percentage grade of 70 or less.

PERCENTAGE OF ACIDITY IN GOOD SILAGE.—With no supplement the percentage of acidity in silage made from fresh alfalfa was 1.646; from wilted alfalfa, 2.522; and from wilted alfalfa plus water, 2.302. With sound corn as a supplement, the average percentage of acidity in silage made from fresh alfalfa was 2.387; from wilted alfalfa, 3.151; and from wilted alfalfa plus water, 3.103. With germinated corn as a supplement, the average percentage of acidity from all bottles in silage made from fresh alfalfa was 2.807; from wilted alfalfa, 3.231, and from wilted alfalfa plus water 3.266. The smaller proportions of corn used show a tendency to produce smaller percentages of acidity. This is shown by the individual figures given in the tables. This statement holds for both sound and germinated corn.

With molasses as a supplement, the average percentage of acidity in silage made from fresh alfalfa was 2.392; from wilted alfalfa, 3.282 and from wilted alfalfa plus water 3.191. As with corn, the smaller amounts of molasses used produce a slightly smaller percentage of acidity.

With rye as a supplement, the average percentage of acidity in silage made from fresh alfalfa was 2.369; from wilted alfalfa, 2.509; from wilted alfalfa plus water, 2.548.

The silage from wilted alfalfa had in all cases a higher percentage of acidity than the silage made from fresh alfalfa. The increase due to wilting varies from about 15 per cent where germinated corn was used to about 50 per cent where no supplement was used. The addition of water to the wilted alfalfa had a slight tendency to reduce the acidity.

PERCENTAGE OF ACIDITY IN BAD SILAGE.—The percentage of acidity in bad silage varies so much that averages have little value. In general the poorest silage had the lowest percentage of acidity, many samples of very bad silage having an acidity value of about 0.5 per cent.

METHOD OF DETERMINING NITROGEN IN AMINO FORM.—The nitrogen in amino form was determined in the same solution used for the acidity determinations, using the formol-titration method according to Sörensen, as described by Jessen-Hansen.[1] The method adapted to this work on silage is as follows: To 25 c. c. of the neutralized alcoholic extract representing 5 gm. of silage, were added 25 c. c. of a neutral solution of dilute formaldehyde. In practice the determination of nitrogen in amino form is made on the same portion of solution as used for acidity. The formaldehyde solution was made by diluting one volume of 40 per cent commercial formaldehyde with one and one-half volumes of water, and making it neutral to phemolphthalein. After adding the formaldehyde, the solution was allowed to stand for 15 minutes, after which the titration was made to a rose-red. In a previous paper[2] attention has

[1] JESSEN-HANSEN, H. DIE FORMOLTITRATION. *In* ABDERHALDEN, Emil. HANDBUCH DER BIOCHEMISCHEN ARBEITSMETHODEN. Bd. 6, p. 262–277, fig. 53. Berlin, 1912.

[2] SWANSON, C. O., and TAGUE, E. L. A STUDY OF CERTAIN CONDITIONS WHICH AFFECT THE ACTIVITY OF PROTEOLYTIC ENZYMES IN WHEAT FLOUR. *In* Jour. Amer. Chem. Soc., v. 38, no. 5, p. 1098–1109. 1916.

been called to the fact that in some extracts like those from flour it is necessary to use thymolthalein. With the extracts from silage there was no difficulty in using phenolphthalein. In the same paper was also discussed the necessity of titrating to a rose-red instead of only to a pink.

The results are given in cubic centimeters of a *N/20* sodium hydroxid, neutralized by the extract representing a 5-gm. charge of silage, and also in percentage of amino nitrogen. This amino nitrogen was calculated on the assumption that 1 c. c. of a *N/20* sodium-hydroxid solution is equivalent to 0.7 mgm. of nitrogen. It is assumed that for every amino group fixed by the formaldehyde there is a corresponding carboxyl group set free. The correctness of this assumption depends, of course, on the nature of the nitrogenous compounds which take part in the reaction. As all these data are comparative, the assumptions made are sufficiently accurate for the present purpose.

PERCENTAGE OF NITROGEN IN AMINO FORM IN GOOD SILAGE.—For the sake of brevity this form of nitrogen will be called titrable nitrogen. The percentage of titrable nitrogen in silage made from fresh alfalfa averages nearly 0.2, and there is only a small amount of variation in the silage from the different bottles. The addition of supplements to fresh alfalfa has very little influence on the amount of titrable nitrogen produced, though the addition of a supplement has a tendency to increase the amount. The amount of titrable nitrogen in silage made from wilted alfalfa averages from one-third to twice that found in silage made from fresh alfalfa. The average percentage of titrable nitrogen with the various supplements to fresh alfalfa was as follows: Sound corn, 0.212; germinated corn, 0.214; molasses, 0.214; rye, 0.254. The average percentage of titrable nitrogen with the various supplements to wilted alfalfa was as follows: Sound corn, 0.337; germinated corn, 0.409; molasses, 0.354; rye, 0.358. In silage made from wilted alfalfa plus water the percentage of titrable nitrogen is notably lower than when water was not used. The percentages in wilted alfalfa plus water with the various supplements were as follows: Sound corn, 0.338; germinated corn, 0.348; molasses, 0.314; rye, 0.334. There is a remarkable uniformity in the amount of titrable nitrogen obtained from the different bottles. The addition of the supplements does not have nearly as much influence as wilting. The addition of water has a tendency to decrease the amount of titrable nitrogen formed.

PERCENTAGE OF NITROGEN IN AMINO FORM IN BAD SILAGE.—Under the same conditions of silage-making the percentage of titrable nitrogen in bad silage is only slightly greater than in good silage. However, the comparison must be limited to the alfalfa used in the same condition. The amount of titrable nitrogen in bad silage made from fresh alfalfa is not as large as the amount in good silage made from wilted alfalfa. The

increase in titrable nitrogen in bad silage as compared with the good is very slight, and in some cases is nil.

COMPARISON BETWEEN THE AMOUNT OF ACIDITY AND TITRABLE NITROGEN.—This comparative study can best be made by noting the number of cubic centimeters used in the acidity and in the formol titrations. In good silage the number of cubic centimeters used in the acidity titration is always greater than the quantity used in the formol titration, and the difference is greatest in the best silage. Where the acidity titration is equal to or less than the formol titration, the quality of the silage is poor. In the very bad silage the decrease in acidity is very large, while the increase in titrable nitrogen is comparatively small, if any. In bad silage it is not the increase in titrable nitrogen that is most notable, but the decrease in acidity. This decrease in acidity is due to molds using up the acids, and also to neutralization of acids by basic nitrogenous compounds. This latter will be discussed further.

RATIO OF TOTAL NITROGEN TO NITROGEN IN AMINO FORM.—The large amount of titrable nitrogen in relation to the total is remarkable. The ratio between titrable nitrogen and total nitrogen in good silage made from fresh alfalfa is 1 to 3+, and in good silage made from wilted alfalfa it is 1 to 2+. In bad silage the amount of titrable nitrogen is almost as large as the total. In the very bad silage nearly all the nitrogen is in this form. This is not due so much to the relative increase of titrable nitrogen as to loss of total. In the splitting of the protein there is an absolute loss of nitrogen. In this experiment there was no attempt to make a careful measurement of this loss; but to judge from the figures obtained, it amounts to about one-third to one-half of the total nitrogen in bad silage.

PART II.—EXPERIMENTS WITH THE ALFALFA SILAGE MADE IN 10-TON SILOS[1]

The seven silos were filled in the spring of 1915 about the time the first cutting of alfalfa was in one-tenth bloom. Corn chop, molasses, sweet-sorghum butts, and rye were used as supplements. The seven silos were filled as follows: No. 1, alfalfa alone; No. 2, alfalfa and molasses, 23 to 1; No. 3, alfalfa and molasses, 10 to 1; No. 4, alfalfa and corn chop, 9.6 to 1; No. 5, alfalfa and sweet-sorghum butts, 5.2 to 1; No. 6, alfalfa and rye, 2 to 1; No. 7, rye alone. The sweet-sorghum butts were of poor quality, and this accounts for the unsatisfactory results obtained; but, even as it was, the silage was of better quality than that obtained where rye was used as a supplement or when alfalfa was used alone. The palatability test made by the Dairy Department showed that the feeding quality of the silage produced ranged in the following order, beginning with the best: Alfalfa and molasses, 23 to 1; alfalfa and molasses, 10 to 1; alfalfa and corn chop, 9.6 to 1; alfalfa and sweet-sorghum butts, 5.2 to 1; alfalfa

[1] The work done in 1914 on this phase is omitted entirely from this paper.

and rye, 2 to 1; alfalfa alone; and rye alone. Alfalfa and corn, and alfalfa and molasses made satisfactory silage for dairy cows. The sweet-sorghum butts as a supplement would no doubt have made excellent silage if they had been first class. The rye proved unsatisfactory as a supplement.

In the center of each silo was placed a telescopic can holding from 25 to 50 pounds of material. The construction of these cans was such that the silage in the cans was subjected to the same pressure as the rest of the silage.[1] On opening it was found that the silage in each can was better than the silage next to the can in the silo. This proves that rigid exclusion of air is of prime necessity, but such rigid exclusion of air is not possible under practical conditions.

CHEMICAL ANALYSES

Samples for chemical analyses were taken by boring into the side of the silo with a 2-inch auger and then removing a core of silage with a 1¾-inch auger. The boring extended to about the center of the silo, and gave a sufficiently large sample for chemical work. This method of sampling is not satisfactory, though it is probably the best that can be used under such circumstances. This is especially true when the silage is made of a mixture of alfalfa and some supplement. Then, when the silage is partly spoiled in places, there is no certain way to ascertain whether an undue proportion of the bad silage is included in the sample. In addition to this, the leaves and stems differ very much in composition, and the right proportion of these is not always obtained. This difficulty in obtaining a satisfactory chemical sample should be considered when the chemical data are studied. But for this difficulty in sampling, the results would show more uniformity in variation.

The plan was to take samples from each silo every day after filling for the first seven days, every other day for the next week, then every four days for the next two weeks, then once a week, and finally once a month. The samples were prepared for analysis by passing them through a clover cutter whose feed was so regulated as to cut lengths ⅛ inch and less. This reduced the material to a satisfactory degree of fineness for all analyses made of the silage in the condition in which it came from the silo. The total nitrogen, as well as the complete feed analysis, was determined on the dried, finely ground samples. The following determinations were made on the fresh samples: Moisture, total acidity, sugar, and nitrogen in amino form as determined by the formol-titration method. These results are tabulated in Table III.

[1] These cans were designed by Prof. J. T. Willard about 25 years ago, while making silage experiments at that time.

TABLE III.—*Chemical changes in alfalfa silage in 10-ton silos*

SILO I (ALFALFA ALONE).

Period.	Moisture.	Total nitrogen.	Amino nitrogen.	Total acidity as lactic acid.	Sugar.
Days.		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0	62.75	1.02	0.0343	0.450	1.039
1	69.25	.91	.0532	.531	.765
2	69.00	.91	.0875	.621	.710
3	64.50	1.04	.1036	.797	.820
4	64.00	1.04	.1190	.936	.629
5	63.75	1.07	.1344	.963	None.
7	66.75	1.03	.1701	1.174	.300
9	63.85	1.04	.1358	1.062	.820
11	67.40	.90	.2338	1.714	None.
14	65.40	.96	.2527	1.629	.259
16	66.20	.73	.1708	1.476	.396
18	67.25	.52	.2394	1.033	None.
21	66.75	Lost.	.1673	1.279	.314
25	66.40	.89	.1813	1.152	.242
29	67.70	.87	.2338	1.845	None.
35	68.85	.84	.2800	1.431	.....
39	70.00	.64	.2176	1.183	.....
42	70.90	.75	.2415	1.260	.....
72	70.30	.75	.2590	1.935	.....
115	72.20	.61	.2030	1.674	.....
163	68.80	.79	.2485	1.562	.....
211	63.35	.96	.2180	1.728	.....
Can	69.95	.73	.2408	1.557	.....

SILO II (ALFALFA AND MOLASSES, 23 TO 1).

Period.	Moisture.	Total nitrogen.	Amino nitrogen.	Total acidity as lactic acid.	Sugar.
Days.		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0	70.83	0.76	0.0343	0.394	1.157
1	78.15	.52	.0658	.927	1.475
2	81.25	.49	.0466	.333	1.940
3	72.15	.75	.1078	.720	1.560
4	73.55	.64	.1008	1.724	.286
6	74.00	.66	.1575	1.886	.164
8	71.35	.71	.1708	2.446	None.
11	72.25	.71	.1624	2.403	.....
13	72.40	.70	.1834	2.441	.....
15	73.00	.68	.1701	2.470	.....
18	73.05	.66	.1568	2.241	.....
22	72.55	.56	.2065	2.538	.....
26	71.75	.75	.1904	2.808	.....
32	73.00	.71	.1932	2.443	.....
34	72.20	.74	.2170	2.448	.....
39	72.10	.74	.2114	2.704	.....
69	72.40	.71	.2100	2.444	.....
112	74.90	.65	.2506	2.452	.....
160	70.40	.73	.2296	2.241	.....
208	71.10	.79	.2814	2.898	.....
Can	74.60	.69	.2828	3.411	.....

SILO III (ALFALFA AND MOLASSES, 10 TO 1).

0	64.85	0.85	0.0394	0.389	.....
1	55.90	1.21	.1050	.702	0.589
2	61.35	.89	.1316	2.394	.900
3	58.45	.91	.1351	2.056	.600
5	61.80	.99	.1953	2.808	.410
7	59.00	1.03	.2212	2.808	.477
9	63.75	.86	.1736	3.015	.205
12	58.65	1.10	.2576	3.123	.273
14	62.40	.92	.1932	3.204	.437
17	63.00	.88	.1554	3.042	.714
21	61.30	.94	.2135	3.172	.442
25	61.50	.87	.1687	2.965	.321
29	65.40	.89	.1582	3.069	1.340
33	64.00	.85	.1806	3.024	None.
38	64.60	.86	.1764	3.172	.850
68	62.00	.87	.2408	2.813	None.
112	67.20	.82	.2436	3.745	.....
158	63.40	.94	.1470	3.348	.....
206	62.70	.88	.1988	3.556	.....
Can	70.40	.79	.2184	2.853	.....

SILO IV (ALFALFA AND CORN CHOP, 9.6 TO 1).

0	66.80	0.84	0.0406	0.378	0.900
1	66.35	.77	.0980	1.212	.123
2	69.60	.68	.1344	1.283	.164
3	67.15	.74	.1778	1.521	None.
5	65.00	.81	.2058	1.836	.....
7	66.15	.88	.2086	1.809	.....
10	67.40	.72	.2086	1.755	.....
12	66.40	.71	.2128	1.791	.....
15	66.60	.73	.2184	1.954	.....
19	65.50	.71	.2268	1.827	.....
23	67.20	.76	.2436	2.173	.....
27	68.30	.74	.2408	2.254	.....
31	67.80	.79	.2492	2.250	.....
36	66.70	.78	.2436	2.286	.....
66	68.10	.76	.2695	2.376	.....
110	69.50	.72	.3430	3.357	.....
156	65.50	.78	.2709	3.078	.....
204	66.70	.82	.2898	3.366	.....
Can	63.40	1.29	.3220	2.988	.....

SILO V (ALFALFA AND SWEET-SORGHUM BUTTS, 5.2 TO 1).

0	64.75	0.91	0.0350	0.387	0.696
1	67.40	.90	.0945	.618	.423
2	67.15	.87	.1540	.972	None.
3	66.50	.86	.1660	1.161	Do.
4	63.35	.77	.2142	1.584	.123
6	68.25	.76	.2562	1.273	None.
8	60.10	.89	.2506	1.768	.....
10	66.80	.81	.2520	1.521	.....
12	68.50	.73	.3038	1.498	.....
15	65.00	.81	.2926	1.728	.....
19	63.40	.88	.2870	1.949	.....
23	63.50	.84	.2961	1.890	.....
28	65.80	.83	.3388	1.759	.....
58	66.00	.84	.3136	1.913	.....
92	56.40	.81	.3325	2.412	.....
150	52.00	1.20	.3038	2.646	.....
198	57.65	.99	.3332	2.196	.....
Can	68.40	.65	.3780	1.485	.....

SILO VI (ALFALFA AND RYE, 2 TO 1).

0	63.25	0.96	0.0376	0.495	3.170
1	66.65	.83	.1127	.859	.629
2	66.60	.80	.1246	1.395	.368
3	69.75	.63	.1491	1.885	None.
5	66.25	.76	.1827	1.863	.....
7	66.75	.73	.2100	1.845	.....
9	69.60	.64	.2296	1.777	.....
11	65.75	.78	.2513	2.097	.....
14	68.50	.36	.2366	1.759	.....
18	62.25	.98	.3493	2.106	.....
22	68.90	.63	.2184	2.133	.....
27	69.80	.66	.2562	1.858	.....
57	69.40	.65	.2380	2.021	.....
91	72.40	.52	.3164	2.435	.....
150	64.80	Lost.	Lost.	1.971	.....
198	67.80	.75	.3129	2.502	.....
Can	61.05	1.00	.3724	2.646	.....

TABLE III.—*Chemical changes in alfalfa silage*—Continued

SILO VII (RYE ALONE).						SILO VII (RYE ALONE).					
Period.	Moisture.	Total nitrogen.	Amino nitrogen.	Total acidity as lactic acid.	Sugar.	Period.	Moisture.	Total nitrogen.	Amino nitrogen.	Total acidity as lactic acid.	Sugar.
Days.		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Days.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0.....	62.25	0.60	0.0350	0.450	1.87	18.....	63.55	.58	.1820	2.619	.....
1.....	64.25	.64	.0932	.680	1.69	22.....	63.90	.56	.1939	2.493	.....
2.....	57.50	.65	.1344	.756	1.64	27.....	61.70	.63	.2198	1.881	.....
3.....	60.40	.63	.1604	1.827	None.	57.....	65.60	.54	.1806	1.665	.....
5.....	59.55	Lost.	.1680	1.720	.83	91.....	67.50	.45	.2440	.774	.....
7.....	60.55	.62	.1764	1.683	.65	150.....	60.90	.63	.1743	2.313	.....
9.....	61.35	.60	.1715	1.584	.80	198.....	62.10	.63	.2352	1.938	.....
11.....	61.05	.65	.2016	2.475	.37	Can.....	57.70	.63	.2212	2.259	.....
14.....	64.10	.63	.1820	1.935	None.						

MOISTURE CONTENT.—The moisture content was highest in the silage made from alfalfa and molasses 20 to 1. In the others the moisture content was practically the same or a little lower than in silage made from well-matured corn or kafir. The moisture content depends on the amount of wilting before the alfalfa is put in the silo, and the moisture content of the supplement.

CONTENT OF TOTAL AND AMINO NITROGEN.—The nitrogen in amino form or titrable nitrogen was determined by the formal titration method, as described in the preceding pages. In all cases there was a sharp increase in the amount of titrable nitrogen in the material after it had been in the silo one day, as compared with the amount present before it was put into the silo. After the first day the increase in titrable nitrogen was gradual, up to the tenth or fourteenth day, when the maximum was reached. The amount fluctuated after this time, but this was probably due to variation in the samples. The silage made from sweet-sorghum butts had the largest amount of titrable nitrogen. The percentage amount of titrable nitrogen in the silage from these seven silos was approximately the same as in the silage made from fresh alfalfa put up in the bottles, but it was much less on the average than that made from wilted alfalfa put up in bottles. There was about the same ratio between total and titrable nitrogen that was found in the silage in the bottles—that is, nearly one-third of the total nitrogen was in the amino form.

TOTAL ACIDITY.—The total acidity was determined in the alcoholic extract as described in the preceding pages and the results calculated as lactic acid. The maximum amount of acidity was reached in approximately the following number of days: Alfalfa alone, 14 days; alfalfa and molasses 20 to 1, 8 days; alfalfa and molasses 10 to 1, 12 days; alfalfa and corn chop, 110 days; alfalfa and sweet-sorghum butts, 100 to 150 days; alfalfa and rye, 11 days; rye alone, 27 days. It should be noted that in those cases where the maximum was reached in the longer time a comparatively large amount of acidity was developed in about two weeks. Also, when the maximum acidity developed in the longer

time, the maximum was larger. This was not due to transfer of acid from above, as the acidity of the silage in the cans was in all cases very nearly the same as the maximum, and in some cases larger.

One function of the acid in silage is to furnish an environment unfavorable to putrefactive organisms. If easily fermentable carbohydrates such as are found in molasses or sweet-sorghum butts are present, the needed amount of acid will be developed through the action of beneficial organisms. The starch in corn is changed more slowly. In alfalfa alone the total amount of acidity produced is too small to bring about the desired condition. Furthermore, silos are not perfectly air-tight, like the bottles used in the preliminary work. Entrance of air makes it possible for putrefactive bacteria to grow.

SUGAR CONTENT.—Sugar was determined in the same alcoholic extract as was used in the acidity determination. The percentage of sugar varies among the different samples obtained from the same silo, but they all have this in common: In a few days to four weeks the sugar disappears. In corn, rye, and sweet-sorghum butts the sugar disappears in the shortest time. This must mean that the soluble carbohydrates are at once used in the production of acid. Where molasses was used in the largest amount, the sugar lasted the longest. More molasses than needed was used. In alfalfa alone the sugar also lasted long. This would appear to indicate that the conditions for transforming the carbohydrates in alfalfa into acids were not favorable.

VOLATILE ACIDS.—Volatile acids were determined according to the method of Dox and Neidig.[1] While the total acidity was calculated as lactic, the volatile acidity was calculated as acetic. By omitting the results for the first few days the following calculated averages were obtained for the total and volatile acidity:

Silage.	Total acidity.	Volatile acidity.
Alfalfa alone	1.483	1.038
Alfalfa + molasses, 20:1	2.413	.958
Alfalfa + molasses, 10:1	3.009	1.119
Alfalfa + corn chop, 10:1	2.242	1.229
Alfalfa + sweet-sorghum butts, 6:1	1.856	1.126
Alfalfa + rye, 2:1	1.975	.750
Rye alone	1.917	.569

Alfalfa alone produced the largest relative amount of volatile acidity. Rye produced a low amount. In the best silage the relative amount of volatile acid was about one-half of the percentage of total. The volatile acidity increased, but the rate of increase was not regular, nor does it seem to have any definite relation to changes in total acidity.[2]

[1] DOX, A. W., and NEIDIG, R. E. THE VOLATILE ALIPHATIC ACIDS OF CORN SILAGE. Iowa Agr. Exp. Sta. Research Bul. 7, 32 p. 1912.

[2] The cause for the relatively large figure for volatile acidity in alfalfa alone will be explained in the fuller report to be published.

SUMMARY

In this paper are presented the results of two series of experiments in making alfalfa silage. One was of preliminary nature, and milk bottles were used as silage containers, and in the other, 10-ton experimental silos were used. Conclusions presented in this paper are based on the results obtained from the bottles as well as from the experimental silos.

Alfalfa silage can be made from alfalfa alone if the containers insure absolute exclusion of air and retention of carbon dioxid. Such conditions are not practical of realization. The addition of supplements insures a more rapid and plentiful production of acids. These make conditions for putrefactive organisms unfavorable.

Wilted alfalfa is more suitable for making silage than the unwilted. The results show that the addition of water to unwilted alfalfa was harmful. Water added to wilted alfalfa gave no decisive results.

Molasses was the most effective supplement. Germinated corn, pound for pound, is more effective than sound corn as a supplement to alfalfa silage. Germinated corn produces results very similar to molasses. Rye would be suitable as a supplement but for the strong odor due to the rye.

Tightness of packing is a condition of success only in that it makes exclusion of air more certain.

Alfalfa silage contains a large amount of nitrogen in amino form. In good silage about one-third of the nitrogen is in this form, and in bad alfalfa silage the amount is sometimes one-half of the total nitrogen.

Most of the acids present in alfalfa silage are produced in the first two weeks. The percentage of acidity may increase after that, but the increase in comparatively slight.

The alfalfa as it is put into the silo contains only a small amount of nitrogen in amino form. Most of the change of nitrogen into amino form takes place in the first 10 days. Silage from wilted alfalfa contains more nitrogen in this form than that made from fresh alfalfa.

Sugar present in the materials used in making silage disappears very rapidly. Completely matured silage contains no sugar.

STUDIES ON OAT BREEDING—V: THE F_1 AND F_2 GENERATIONS ON A CROSS BETWEEN A NAKED AND A HULLED OAT[1]

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INTRODUCTION

The present paper is an account of the results obtained from a cross between representatives of two subspecies, the naked oat, *Avena sativa nuda* var. *inermis* and *Avena sativa patula* var. Victor. These varieties possess several contrasting characters. An examination of the literature has failed to reveal any detailed descriptions of the inheritance of the naked and hulled characters in oats. Von Tschermak (9, p. 85; 10, p. 364)[2] states that in a cross between a naked variety, *Avena chinensis*, and a hulled oat the hull-less character is apparently dominant. The segregation resulted in naked forms, intermediate forms, and fully hulled oats. He also states that the multiflorous character of the naked oat is correllated with the naked character of the grain and that the character of firmly hulled grain excludes the multiflorous condition.

It seemed probable that a more detailed study of these and other characters would be of considerable interest. The present paper reports the results obtained as far as the second generation. The main characters dealt with in this article are the type of flowers and hulls and the characters of the glumes (pubescence, color, awning). This cross was made in 1914, and the progeny in both generations was grown out of doors. In the absence of the data on the third-generation plants, certain questions arising in the discussion could not be fully solved. The great variety of intermediate forms segregating in the second generation is responsible for the fact that a large part of this paper is descriptive. The more or less minute description of the parents and their progeny was also prompted by the desire to give a detailed picture of the forms involved, in order to facilitate the establishment of their botanical identity.

Regarding the methods used, the reader is referred to the paper recently published by one of the writers (6).

THE NAKED PARENT, AVENA SATIVA NUDA

The seed of the naked parent used in this cross was secured from the Russian Bureau of Acclimatization, having been originally grown in the Experimental Garden of the Horticultural School at Cholmy, Russia.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station: No. 112.
[2] Reference is made by number to "Literature cited," p. 311–312.

Journal of Agricultural Research,
Washington, D. C.
jd

Vol. X, No. 6
Aug. 6, 1917
Key No. Me.—11

The hull-less seed was received under the name "*Avena chinensis*—China oat." However, the study of the plants grown from that seed in the breeding garden of this Station has led to the observation of some details which renders the correctness of the name under which the seed was received from Russia very doubtful. From the following description of the plant the conclusion seems justified that the variety in question is not *Avena sativa nuda* var. *chinensis*, but *Avena sativa nuda* var. *inermis* Kcke.

When grown in garden rows and under conditions prevailing in Maine, the plants attain an average height of about 130 cm. The stooling is medium, the average number of culms per plant being five. Plate 39, *A*, shows the general characters of the panicle. The head is about 30 cm. long. The ascending branches form a rather sharp angle with the main axis of the panicle, and give the head an oblong appearance.

The most interesting feature of this oat is the structure of its spikelets. While the number of kernels per spikelet in the common oat ranges from two to three, the spikelets of the naked oat are multiflorous, the number of flowers or kernels ranging from three to five. This feature appears in the most pronounced form at the tips of the whorls. Here, as a rule, the spikelets contain five flowers or kernels, while with those spikelets in the lower part of the whorl and also in the lower part of the panicle, as a whole, the number of flowers per spikelet is reduced so that in certain panicles the lowest whorls bear spikelets with only two to three flowers. Plate 40, *A*, shows a typical spikelet of a naked oat.

The long pedicels (rachis) of the individual flowers give the spikelet a raceme-like appearance. The length of the pedicels bearing the individual flowers varies considerably within the same spikelet, increasing from the uppermost flower towards the lowest one at the base of the spikelet. The relative length of the successive pedicels in a pentaflorous spikelet, from the uppermost down to the lowest, averages about 3.8, 6.0, 8.7, and 12.2 mm., respectively. The average length of the pedicels bearing the upper flower in an ordinary hulled oat averages about 2.5 mm., the shortest pedicels within a spikelet of a naked oat thus being still longer than the average length of the pedicel in the spikelet of the common oat.

It is of interest to note that the absolute length of the pedicels in the different spikelets of a panicle is also subject to variation. The uppermost spikelets of the main axis of the panicles, as well as the uppermost spikelets of each whorl, show the greatest absolute length of the pedicels, and consequently the longest spikelets. In the spikelets situated nearer the base of a whorl the pedicels have a tendency to be reduced in length. The same is true of the panicle as a whole. There is a gradual reduction of the absolute length of the pedicels and spikelets

toward the base of the panicle. Along with the reduction of the length of the pedicels, there occurs also a reduction of the number of flowers or kernels within the individual spikelets from the top of the main axis toward the base of the panicle. The same is true, but to a less extent, for each whorl. The tip spikelets of a given whorl are always the longest spikelets, and contain more flowers than any other spikelets in that whorl. Near the base of each whorl the spikelets show a marked reduction both in length and in number of flowers. This reduction of the number of kernels in the spikelets at the base of the whorl and of the panicle in conjunction with the shortening of the pedicels gives the spikelets in those regions of the head almost the appearance of those on a common hulled oat.

The reduction in the size and number of the spikelets which takes place gradually from the top to the base of the panicle and of each whorl recalls the well-known fact that the heaviest kernels in the oat panicle are borne near the top of the head and at the tips of the whorls. The lightest kernels are found at the base of the whorls and of the panicle, where sterility of the flowers quite often occurs. It is probable that the same physiological causes that favor the development of the grain in the uppermost regions of the common oat head account also for the larger number of flowers or kernels and for the greater length of the pedicels in the uppermost spikelets of the naked oat.

Another interesting feature of the spikelets of the naked oat is presented by the nature of the glumes. A multiflorous spikelet of the naked oat used in this cross bears at its base a pair of yellowish outer empty glumes, the glumæ proper, which cover the flowering glumes, or rather the paleæ. The lower flowering glume, or the palea inferior, is slightly longer than the corresponding outer sterile glume. The upper, inner glume, the palea superior, however, is much shorter, reaching only to about the middle of the palea inferior (Pl. 41, *B*). The length of the lower and upper flowering glume averages about 22.7 and 13.3 mm., respectively. This proportion between the size of the lower and upper flowering glume, characteristic of this naked oat, obtains also in the remaining flowers of the multiflorous spikelet. The outer flowering glume sometimes bears a very weak, straight awn. Whenever the awns are present, they are borne chiefly by the spikelets on the lower whorls. The rare occurrence and weak nature of the awns suggest that the strain used in this cross belongs to the variety *Avena nuda* var. *inermis*.

The structure of the lower flowering glume is very characteristic of the naked oat. As pointed out in another paper by one of the present writers (12), the lower flowering glume of the common oat represents a very strong protective organ of the flower and fruit of the Gramineae, being composed of four layers of cells, two layers of which, thickened and reenforced by a deposition of silicious matter and silicious cells, represent

the strong mechanical system of the lower flowering glume (Pl. 41, *A*). In the case of the naked oat there is no such differentiation of the cells of the lower flowering glume. As can be seen from Plate 41, *B*, the lower flowering glume is quite thin and membranous; it is also unusually wide and long. Its structure does not differ from that of the sterile outer glumes. This weak structure of the lower flowering glume of the naked oat, its unusually large dimensions in conjunction with the fact that it does not adhere to the caryopsis at all, gives to it the appearance of a sterile glume similar to the outer, empty glumes.

The color of the glumes is pale yellow. The caryopsis is slender and of light-yellow color (Pl. 42, *C*). The nakedness of the kernels, the multiflorous habit of the spikelets, the white color and absence of awns, are the main characters of the naked oat involved in the cross dealt with in this paper.

THE HULLED PARENT, AVENA SATIVA PATULA

The common hulled parent oat crossed with the naked oat is a pure line, known in our record books as line 262, and was selected from a variety known as the Victor. This variety is a black oat bred by a commercial seed company. According to its catalogue, this oat "was produced from six different parents, two of which were fall oats." Notwithstanding its hybrid origin, this variety in general and line 262 in particular breeds perfectly true in all characters. Line 262 was isolated in 1910 and has been grown every year since. A description of the Victor variety has been given by Surface and Barber (7). Line 262 was one used in the selection work carried on by Surface and Pearl (8).

The Victor oat is characterized by a tall straw. Its height in the garden averages about 155 cm. As seen in Plate 39, *A*, the head possesses a very long axis, long thin, drooping branches, covered with a moderate number of spikelets. Toward maturity the slender wavy branches yield to the weight of the grain, thus intensifying the drooping appearance of the head.

The grain of the Victor oat, as seen in Plate 46, *A*, is of medium size, the caryopsis being firmly inclosed by the flowering glumes. The color of the grain varies from dark brown to black. The lower grain bears a medium strong, kneed, and twisted dark-brown awn. The base of the grain is marked by a rather wide cleavage plane, similar almost to the base of the intermediate type resulting from a cross between a wild (*Avena fatua*) and a cultivated oat. On the majority of spikelets a few rather long hairs may be found at the sides of the base of the lower grain (Pl. 46, *A*). The main characters of the Victor oat contrasting with those of the naked oat are the hulled kernel, black color of grain, biflorous habit, the rather heavy awns, and the pubescence at the side of the base of the grain.

FIRST GENERATION

The first generation originated from a cross in which the naked oat was the male parent and the Victor oat the female. Eleven hybrid grains were obtained, seven of which failed to germinate when planted in the garden in 1915. The plants arising from the remaining four grains exhibited, on the whole, uniform characters, there being only a slight quantitative variation of the hull character. This point will be referred to below. Plate 39, *C*, gives the external appearance of the F_1 plants. By comparing this figure with figures *A* and *B* of Plate 39, it may be noted that the F_1 plants present an intermediate type between the two parents as to shape of head and form of spikelets. The longer head and the more widely spreading branches of the F_1 plants markedly contrast with the type of head of the naked oat and resemble rather the Victor parent, though the two types can be easily distinguished from each other.

With regard to the multiflorous characters, the F_1 plants show a prevalence, if not dominance, of this character over the common biflorous habit of the Victor oat. This observation agrees with the results obtained by Von Tschermak (9, p. 85; 10, p. 364) in a cross between the multiflorous oat *Avena sativa* var. *chinensis* and a common oat with normal flowers. While the habit of the spikelets of the F_1 plants is generally multiflorous, their form is different from that of the spikelets of the naked parent. This can be clearly seen by comparing figures *B* and *C* of Plate 39. The pedicels of the individual flowers within the spikelets of the F_1 plants are shorter than in the case of the naked parent, thus causing a shortening of the multiflorous spikelet. It was stated above in connection with the description of the naked oat that there is a tendency for the pedicels and for the flowers to be reduced in length and number, respectively, from the top of each whorl, and also from the top of the panicle as a whole toward the base. This tendency is still more pronounced in the F_1 plants. The longest multiflorous spikelets are grouped on the tips of the upper whorls and lead through gradual transitional forms to the biflorous spikelets present at the base of the whorls and of the panicle. It will be worth while bearing this feature in mind, as it is shown in a still more pronounced form in the intermediate types of F_2 plants and is correlated with gradual changes in the nature of the glumes.

With regard to the inheritance of the hull character—that is, the way in which the flowering glumes inclose the caryopsis—the F_1 plants represent an intermediate condition. Both parental forms, as well as the intermediate types of kernel described below, are present on the same panicle (Pl. 44, *A*). These intermediate forms are rather interesting, as they show naked kernels and firmly hulled ones side by side within

the same spikelet. Thus, spikelets occur in which the lower kernel is naked and the upper intermediately hulled. Another form is characterized by both lower and upper kernels being intermediately hulled. In still another type which occurs more frequently than either of the others the lower kernel is intermediately hulled and the upper completely hulled.

The intermediately hulled kernel is characterized by the following features: The caryopsis is inclosed by the lower flowering glume, which is for the most part membranous, hardening only along the median vascular bundle (Pl. 43, *C*). It is along this dorsal line that the glume adheres slightly to the caryopsis. The lateral edges of the glumes stand off freely, without embracing the caryopsis. The upper flowering glume lies closer to the caryopsis than in the case of the naked oat, but does not adhere to it.

All the types of hulls given above appear within the same panicle; in fact, often in the same whorl. As to the numerical distribution on the F_1 panicle of spikelets with different types of hulls, the spikelets with firmly hulled kernels and the naked ones occur in about the same numbers, while the intermediate types taken together exceed the total number of the former two groups. Since the intermediate types of spikelets are composed of both hulled and naked kernels, it may be said that the naked grain is prevalent over the hulled grain.

The local distribution of spikelets with varying types of hulls over the panicle is of some interest. As an illustration, the distribution of spikelets on one panicle may be presented here. All the spikelets of the head were examined, beginning from the top spikelet of the uppermost whorl downwards to its base. This order was maintained in the case of each whorl. The figures in Table I indicate, in numerical succession for each whorl, which of the successive spikelets, beginning with the uppermost, presented a given type of hull.

From this table the following points may be noted: The spikelets in which all the kernels are naked or in which the type of hulls most nearly approach that condition are grouped on the upper branches of each whorl. On the lower branches of each whorl the naked kernels gradually become replaced by transitional forms leading up to the perfectly hulled kernels at the base of the whorl. This is also true of the panicle as a whole. The uppermost whorl bears only spikelets with naked kernels. The next lower whorl already exhibits transitional forms characterized by the appearance of more or less intermediately hulled kernels. With the lowest whorl a condition is reached which is almost the opposite of the uppermost whorl.

If we compare this feature with the behavior of the multiflorous character in the F_1 plants, we can easily note an interdependence between the two characters. The multiflorous spikelets are similarly grouped about the top of the panicle and on the upper branches of each whorl,

reaching a biflorous condition at the base of the panicle and at the base of the whorls. The normal biflorous, or rarely triflorous, condition of the spikelet is completely correlated with the firmly hulled kernels. There is a negative correlation between the tetraflorus or pentaflorous condition of the spikelet and the firmly hulled kernels, as no multiflorous spikelets contain firmly hulled kernels. This negative correlation was also observed by Von Tschermak (9, p. 85; 10, p. 364) in his cross between a common oat and the multiflorous *Avena sativa* var. *chinensis*. It follows, then, that there is a correlation between the multiflorous character of spikelets and the hull-lessness of the kernels. This correlation, however, does not appear to be complete, as some cases were observed in the second-generation plants where naked kernels are borne by biflorous spikelets (Pl. 42, *A*).

TABLE I.—*Distribution of the different types of spikelets on the panicle in F_1 oat plants*

Whorl No.	Type of hulls of grain in individual spikelets.	Position of successive spikelets on whorl.
I	All kernels naked	1, 2, 3, 4, 5.
II	All kernels naked	1, 2, 3.
	All kernels intermediately hulled	4.
	Lowest kernel intermediately hulled; upper firmly hulled	5, 6.
III	Kernels intermediately hulled	1.
	Lowest kernel naked; upper firmly hulled	2, 3, 4, 6.
	Both kernels naked	5.
	Lowest kernel intermediately hulled; upper firmly hulled	7, 8, 9, 10, 11.
	All kernels hulled	12.
IV	All kernels naked	1, 2, 3.
	Lowest kernel intermediately hulled; upper firmly hulled	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15.
	All kernels hulled	16, 17, 18, 19, 20.
V	All kernels intermediately hulled	1, 2.
	All kernels hulled	3, 4, 5.

Regarding the inheritance of the grain color, the F_1 plants show dominance of the black pigment, although the color of the grain is not so intense as the black color of the Victor parent. In connection with the distribution of the pigment it is of interest to note that in the case of a hull-less grain the lower membranous flowering glume shows no pigment whatever, being, as it is, in no connection with the grain. On such grain the pigment is confined to the upper flowering glume. In the same measure, however, as the lower flowering glume begins to grow coarser and adheres along the line of the median vascular bundle, the pigment appears along that line, spreading out from it over the remainder of the hull body with decreasing intensity.

The size of the kernel of the F_1 plants is intermediate between the two parents.

On the F_1 plants the awns are present only occasionally, being borne by the spikelets of the lower whorls. The awns are weak and straight, with no basal portion. The appearance of the awn, as will be seen more distinctly in the F_2 plants, is limited by the nature of the glume that bears it.

The character of pubescence appears in the F_1 plants more distinctly than with the Victor parent. The base of the firmly hulled and intermediately hulled kernels bears at its sides a fairly thick tuft of hairs. Since the pubescence on the grain of the Victor parent occurs only in the form of a few hairs, it would appear that the intensifying of the pubescence of the F_1 plants is possibly due to the influence of hybridization. The part which the other parent, *Avena nuda*, might possibly play in the augmentation of the pubescence can not be easily determined, as its kernels are naked, and, hence, offer no chance for the pubescence to develop. This point will be referred to later in connection with the discussions of the character of pubescence in the second-generation plants.

SECOND GENERATION

The four F_1 plants furnished seed enough to raise a considerable number of F_2 plants in 1916. The second generation comprised 854 plants, all of which were examined for the characters discussed above.

A.—HULL CHARACTER

With reference to the hull characters the F_2 segregation presents a variety of forms, among which at least six types can be distinguished. Two of these types represent the parental forms. Plate 43, *B*, shows the typical naked form from the F_2 generation. A comparison of this figure with figure *B* of Plate 39 shows a slight difference in the appearance of the two types, owing to the fact that in the naked forms from the F_2 generation the pedicels are often somewhat shortened, which causes the spikelets to appear more contracted than in the case of the naked parent. The multiflorous character of the naked forms of the F_2 generation is in several cases also modified in the direction of a reduction of the number of flowers per spikelet, pentaflorous spikelets being confined mainly to the top of the whorls, while the other spikelets contain only four or three flowers. This condition is very pronounced in some plants where the biflorous spikelets with naked kernels prevail over the multiflorous spikelets. A few plants were even found where, except for a few top spikelets with three or seldom four kernels all spikelets are biflorous, with two naked kernels per spikelet. These plants look much like a normal biflorous oat (Pl. 42, *A*). This is interesting to note, as it shows that the correlation between the multiflorous character and nakedness of the kernels is not complete.

The second F_2 type to be described contains the plants with fully hulled grain. This type is much simpler and more uniform than the

hull-less type discussed above. The biflorous condition of flowers is completely linked with firmly hulled kernals. In this respect the F_2 generation segregates a group of plants which reproduce the type of the Victor parent, as will be seen from Plate 43, *A*. The grain of this group, except for the rather heavy pubescence at the base of the grain, resembles very closely the type of grain of the Victor parent (Pl. 46, *B*).

Within the limits drawn by the above two groups of the parental type there is a rather wide range of intermediate forms marked by different gradations in regard to the hull character. Four types may be distinguished. Beginning with the group most nearly approaching the naked condition of grain, there is a form in which a few upper kernels in spikelets borne on the lowest whorl of the panicle are intermediately or firmly hulled, all the others being naked. The next form stands close to the former, but is marked by the presence of a few intermediately hulled lower kernels in the spikelets in the lower region of the panicle.

This form leads to the next type, which represents the largest group among the intermediate plants and shows all possible gradations between the naked and the hulled oat on the same panicle. Plate 45 shows a series of spikelets with the different gradations of the hull character, taken from a single oat head. The spikelets with the naked kernels are borne on the upper portions of the whorl. Next to these appear spikelets in which the lowest kernel is naked, and the upper ones are intermediately hulled. About the middle of the panicle spikelets prevail in which the lowest kernel is naked and the upper kernels firmly hulled. Below these, a condition is reached in which the lower kernel in the spikelet is intermediately hulled and the upper kernels are firmly hulled. Finally, at the base of the spikelet there are a few perfectly hulled kernels. It will be noted that this group of intermediate plants resembles the F_1 generation more closely than any other form.

The last group of the F_2 plants with intermediately hulled grain linking with the type of plants with firmly hulled grain shows a rather simple condition in which the tip spikelets are hull-less, the grain in the middle section of the panicle is intermediately hulled, while the lowest part of the panicle bears only completely hulled grain.

After the description of the F_2 forms as to type of hull, the inheritance of the hull character may now be discussed. As shown above, the first-generation plants are intermediate, showing the characters of both parents. The second generation segregates into two groups of the parental types and into a group of intermediate forms presenting a continuous series of different gradations. Whether these gradations within the group of intermediate forms are controlled by separate factors that will cause some of them to breed true or whether they are to be considered as a result of the heterozygous condition with only one pair of factors involved can not be determined at present. It is hoped that the data for the third generation will throw light on this

question. So far as the segregation in F_2 is concerned, it can be well explained by assuming one pair of genes. The heterozygous condition of these genes is represented by the intermediate plants, while the plants with typically hulled and typically naked grain represent the respective homozygous condition. From the detailed description of the intermediate plants it will be clear that neither gene is truly dominant. In general appearance the intermediate forms more nearly resemble the hull-less parent.

Table II shows a good agreement between the observed and the expected results. All the intermediate forms are here grouped in one class.

TABLE II.—*Segregation in regard to the hull character*

Item.	Hulled.	Intermediate.	Naked.
Observed	221	404	229
Expected	213.5	426	213.5

The inheritance of this character thus exhibits a Mendelian behavior, which is known as the Zea type, after Correns. This condition is analogous to that presented by the base of the lower grain in the cross between a wild and a cultivated oat (6) and by the character of awning in the cross between certain bearded and beardless wheats (1, p. 157–159; 3). The question whether the inheritance of the hull character of the present cross will follow the pure Zea type or the intermediate modification of that type established by Von Tschermak (9, p. 85; 10, p. 364), for the hull character of barley can not be solved until the data for the third generation are available.

B.—GRAIN COLOR

The segregation in regard to the grain color presents a simple Mendelian ratio. As already stated, the F_1 plants show a dominance of the pigment, even though it does not possess the intensity of the color of the Victor grain. In the second generation there is also a group of plants showing a light-brown color of the grain, but the intensity of the pigment grades from the light-brown color over brown to dark brown and black when a condition similar to that of the black parent is reached. Grouping all plants with pigmented grain into one class, we obtain the following ratio:

Observed ..... Black : White = 646 : 208.
Expected ..... Black : White = 640.5 : 213.5.

As will be noted, the observed results agree very well with the expected on a monohybrid ratio.

It will next be of interest to determine whether there is a relation between the factors controlling the hull character and the color genes. In Table III all the intermediately hulled forms are grouped in one class.

TABLE III.—*Relation between the hull character and the grain color*

Item.	Hulled.		Intermediate.		Hull-less.	
	Black.	White.	Black.	White.	Black.	White.
Observed	166	55	296	108	184	45
Expected	*160. 1*	*53. 4*	*320. 3*	*106. 8*	*160. 1*	*53. 4*
Ratio	*3*	*1*	*6*	*2*	*3*	*1*

From this table it is evident that the observed numbers agree fairly well with the expected and show that the genes for the hull character and grain color segregate independently. As mentioned earlier, the intermediate forms show a greater resemblance to the hull-less oats than to the hulled forms. If the plants with intermediate and hull-less grain are grouped together, a still better agreement between the observed and expected numbers is obtained. This is shown in the Table IV, where the expected numbers are calculated on the 9 : 3 : 3 : 1 ratio.

TABLE IV.—*Relation between the hull character and the grain color*

Item.	Black.		White.	
	Hull-less.	Hulled.	Hull-less.	Hulled.
Observed	480	166	153	55
Expected	*480. 3*	*160. 1*	*160. 1*	*53. 4*

C.—PUBESCENCE AT THE BASE OF THE GRAIN

Another character involved in this cross is the pubescence at the base of the grain. The conditions in regard to this character are somewhat complicated by the nature of the naked parent. The character of the pubescence can only be established for the hulled Victor parent and for the hulled progeny, while it is obvious that in the case of the naked parent and all the naked forms of the segregating progeny the manifestation of that character is impossible. Moreover, there is evidence that also in the majority of the intermediate forms of the second generation the total or partial nakedness of the lower kernels interferes with the normal manifestation of the pubescence. This causes a deviation from the expected type of pubescence, which will be discussed below. In grouping the intermediate forms of F_2 in regard to pubescence it is often difficult to decide whether a given plant does not carry the factor for pubescence at the base of the grain or whether its manifestation is only foiled by the fact that the grain is not inclosed in the firm glumes.

In studying the inheritance of this character it may be well to consider first the plants in which the hull character does not interfere with

the free expression of the genes for pubescence at the base of the lower grain if present. In the present data there are 323 F_2 plants which fulfill these conditions. They include all the plants with completely hulled grain and those intermediate forms which most closely approach this condition. Of these plants 300 are pubescent and 23 are smooth (Pl. 42, *B*). These numbers indicate a bifactorial character. Calculating the expected number of plants on the 15-to-1 ratio, we have the results shown in Table V.

TABLE V.—*Segregation with reference to the pubescence at the base of the lower grain*

Item.	Pubescent.	Smooth.
Observed number	300	23
Expected number	302.8	20.2
Ratio	15	1

The agreement here is very good.

It should not be forgotten, however, that these results are obtained from a selected population. In such a case there is always the danger that, through linkage or for other reasons, the selected group may not represent the condition in the whole population. However, a consideration of all the forms of the second generation that have an opportunity for the expression of the genes for pubescence furnishes evidence that the group of plants discussed above does represent a random sample of the F_2 population. Of the whole F_2 population 229 plants have completely naked grain. It is obvious that these plants must be excluded from a consideration of the character of pubescence. Of the remaining 625 plants, 369 are pubescent at the base of the lower grain, 128 are pubescent at the base of both lower and upper grain, 81 are pubescent at the base of the upper grain only, and 47 are smooth. The group of 81 plants pubescent only at the base of the upper grain presents a very interesting condition which is the result of the interference of the nakedness or seminakedness of the lower grain with the manifestation of the pubescence at its base.

While this case will be more fully discussed in connection with the discussion of the pubescence of the upper grain, it may suffice here to state that in all the 81 plants the lower grain was totally or partly naked, and that this condition obviously prevented the development of pubescence at the base of the lower grain. This circumstance, coupled with the fact that heretofore not a single case has been reported of an oat, cultivated or wild, with the lower grain smooth and the upper pubescent, justifies the conclusion that the 81 plants might have developed the pubescence at the base of the lower grain if it were not for its naked or

seminaked condition. Including, then, these plants in the group of plants with pubescence at the base of the lower grain, we obtain the ratio of 578 pubescent to 47 smooth. The expectation on a 15-to-1 ratio is 586.5 to 39.1. The agreement between the observed and expected numbers is fairly good and bears out the conclusion regarding the bifactorial character for pubescence found above for the selected group of completely and almost completely hulled forms. This group then appears to represent a random sample of the F_2 population in respect to pubescence. To avoid the disturbing influence of the naked or seminaked condition, only this group of completely or almost completely hulled forms will be considered in the further discussion.

It will next be of interest to see whether there is any relation between the genes for pubescence at the base of the lower grain and the color genes. Of the 323 plants to be considered, 236 are black and 87 white. This is a very close approximation to the expected 3-to-1 ratio, and shows that in respect to grain color this selected group of plants is representative of the complete F_2 generation.

If there is no linkage between either of the factors for pubescence and the color genes, the expected ratio will be 45 black pubescent to 3 black smooth, to 15 white pubescent to 1 white smooth. The expected numbers calculated from this ratio, together with the observed number of plants in each class, are shown in Table VI.

TABLE VI.—*Relation of the color genes and the genes for pubescence at base of the lower grain*

Item.	Black.		White.	
	Pubescent.	Smooth.	Pubescent.	Smooth.
Observed number	226	10	74	13
Expected number	*227.1*	*15.1*	*75.7*	*5.1*
Ratio	*45*	*3*	*15*	*1*

This is a very reasonable agreement between the observed and expected numbers. These results show that the color genes segregate independently of the two genes for pubescence.

As shown in Plate 46, *A*, the pubescence on the Victor oat is quite long, in fact much longer than the hairs found at the base of most cultivated oats. Nilsson-Ehle (4) has stated that the long and short pubescence at the base of the grain segregates in a monohybrid ratio. This is found to be the case in the present cross. Out of the 300 pubescent plants 217 have long pubescence and 83 short. This is undoubtedly a 3-to-1 ratio.

Furthermore, the genes for long and short pubescence segregate independently of the color genes, as shown in Table VII.

TABLE VII.—*Relation of the color genes and the length of pubescence*

Item.	Black.		White.	
	Long.	Short.	Long.	Short.
Observed number	172	55	44	29
Expected number	165.3	55.1	55.1	18.7
Ratio	9	3	3	1

On returning to the question of the presence and absence of pubescence at the base of the lower grain, several interesting features may be noted. As already stated, the grain of the Victor parent shows a few long hairs at the base of the lower grain. In the first generation this pubescence is intensified, and in the second generation many plants show thick, long tufts at the side of the base. The difference in the pubescence of the grain of the parent plant and these second-generation plants can easily be seen by comparing figures *A* and *B* of Plate 46.

The F_2 generation thus presents an augmentation of the pubescence. The pubescence appears here not only intensified but also as a dihybrid character. The mere intensifying of the pubescence may conceivably be due to the stimulating influence of hybridization. It is further possible that the augmentation of the pubescence and its dihybrid character is accounted for by the hulled Victor parents' bearing latent characters which through hybridization become activated and released.

A more plausible and simple explanation of the bifactorial character of the pubescence in the second generation is suggested by the assumption of a second gene for pubescence entering from the naked parent. The question whether the naked parent contributed a gene for pubescence in the hybrid progeny can not be solved directly, since, owing to the nakedness of the grain, the manifestation of a possible inherent gene for pubescence is prevented. However, the evidence obtained in other crosses between the hulled Victor oat used in the present cross and other hulled strains tends to indicate that the pubescence of the Victor oat is a monohybrid character. This evidence then speaks in favor of the assumption in the present cross of a gene for pubescence in the naked parent.

A still more remarkable feature of this cross is the presence of a pubescence of a distinct type at the base of the upper grain in the hybrid plants (Pl. 46, *B;* 47). While several cultivated oat varieties have been observed which possess a pubescence of varying strength at the base of the lower grain, only three cases are recorded in the literature in which a few individual spikelets developed pubescence also at the base of the upper grain. Thus, Christie (2) records a case of two Danish varieties developing a pubescence at the base of the upper grain. Nilsson-Ehle (5) found that certain plants which he regards as atavistic regressions

toward the wild-oat parent showed a pubescence on the upper grain. These atavistic regressions represent really the wild form. In similar cases observed occasionally by the present writers the pubescence appears not in form of tufts of hair at either side of the base, but covers the whole base as in the wild oat. Finally, Fruwirth (2) observed two spikelets of an oat plant in which also the upper grain had a pubescence. This character, however, did not prove to be heritable, as the progeny of that plant did not develop it.

In many wild oats, *Avena fatua*, *A. sterilis*, etc., a heavy pubescence is developed at the base of the upper grain. In *A. fatua* that condition appears completely correlated with other specific characters (11, 6). The type of pubescence in the present case, however, is different from that of the upper grain in the wild oat. While in the latter case a heavy, thick pubescence covers all sides of the base (6, pl. 3, fig. 6) the pubescence developed at the base of the upper grain in hybrid plants of the cross in question shows only two more or less thick tufts of hair at either side of the base. As seen in Plate 46, *C*, this type of pubescence is similar to that of the intermediate forms resulting from crosses between *A. fatua* and cultivated oats.

In order to determine the distribution of the plants with pubescence on the upper grain in relation to the character of the glume, the plants were grouped in three classes. The first class comprises the plants with typically intermediate type of hull—that is, plants with a majority of intermediately hulled lower grain and a few fully hulled. The middle group represents the forms most closely approaching the hulled condition of grain, while the third class contains only the plants with normal, firmly hulled grain. Table VIII shows these three classes of plants in relation to the pubescence on the upper grain.

TABLE VIII.—*Relation between the pubescence on upper grain and the hull character*

Character of hull	Intermediate.		Intermediate, with prevalence of hulled grain.		Hulled.	
Upper grain	Pubescent.	Smooth.	Pubescent.	Smooth.	Pubescent.	Smooth.
	176	136	33	59	0	221

This table shows that the majority of plants with pubescence on the upper grain fall into the group in which the lower grain is either naked or intermediately hulled and the upper intermediately or firmly hulled. In the same measure as the glume of the lower grain becomes coarser approaching, as in the middle group, the firmly hulled condition, the pubescence on the upper grain tends to disappear.

In the last group with normal, completely hulled grain none of the upper grain develops a pubescence at the base. This gradual change in

the manifestation of the pubescence can be observed on the same panicle, even on the same whorl, in an intermediate form. The top spikelets in this case are usually naked, no manifestation of the pubescence being possible. In the succeeding spikelets the lower grain may be naked and the upper intermediately or firmly hulled, the pubescence, if the gene for it is present, appearing at the base of the upper grain. Eighty-one plants presenting this condition were observed. In the spikelets located still lower on the whorl or panicle the glume of the lower spikelets become coarser, adheres more closely to the caryopsis, in which condition the pubescence appears already on the lower grain and decreases on the upper; finally, the firmly hulled normal condition of the lower grain causes the complete disappearance of the pubescence on the upper grain. Thus, a single panicle or even whorl presents gradual changes in the local manifestation of pubescence, following the changes in the type of hull.

This fact, as well as the evidence obtained from other crosses between the Victor line and common hulled-oat varieties in which no case of an upper grain being pubescent was observed, make it evident that the development of pubescence at the base of the upper grain in the progeny of the present cross is caused by the naked or seminaked condition of the lower grain. Obviously, this naked or seminaked condition of the lower grain causes a disturbance in the normal manifestation of the pubescence, and it seems as though the gene for pubescence, suppressed or restrained in its appearance in the normal region, tends to manifest itself at the base of the upper grain, this latter thus taking the part of the lower grain.

The examination of plants relative to the presence and absence of pubescence at the base of the upper grain does not indicate a simple Mendelian behavior. There are 209 plants with pubescent upper grain and 416 plants with smooth upper grain. The expected numbers are, on a 3-to-1 ratio, 156 pubescent to 469 smooth, the difference between the observed and expected numbers being 53. This difference is five times larger than the standard deviation ($\sqrt{n\,p\,q} = 10.8$) and is probably beyond the range of fluctuation of random sampling.

The development of pubescence at the base of the upper grain in this cross is then plainly the result of the modifications caused by the naked parent. The same cause also accounts for the abnormal behavior of the 81 plants which develop a pubescence at the base of the upper grain while the lower grain is smooth. Already a casual inspection shows that, since in all the 81 plants the lower grain is naked or seminaked, the expression of the gene for pubescence is prevented. This is borne out by the fact that when the lower grain is completely hulled, as in the last group of Table VIII—thus giving full opportunity for developing the pubescence—none of the upper grains are pubescent. No single case has been recorded in the literature of an oat with a smooth lower grain and pubescent upper grain. As already pointed out, only certain wild oats normally develop a pubescence on the upper grain, but even here the development

of that pubescence is controlled by the pubescence of the lower grain and, as Surface (6) showed for the cross between wild and cultivated oats—

in the absence of the gene for pubescence on the lower grain the gene for pubesence on the upper is unable to act.

D.—INHERITANCE OF AWNS

The inheritance of the awn character remains to be discussed. The behavior of this character, like the pubescence discussed above, is closely associated with the morphological constitution of the lower flowering glumes and is affected by the same disturbances caused by the naked parent. As already stated, the strength of the lower flowering glume in the intermediate forms may vary on the same panicle, or even whorl, from a thin membraneous condition to the coarseness and strength of the normal hull. These variations of the glume are accompanied by corresponding variations in the strength of the awn. The naked forms and the naked spikelets of the intermediate forms bear only a very weak, thin awn, often appearing as a tender prolongation of the median, vascular bundle at or near the tip of the glume. The group of intermediate forms approaching the firmly hulled forms have partly a weak awn and partly a medium strong awn, with a distinct twisted, basal portion. The third group, including only plants with firmly hulled grain, possesses a strong kneed and twisted awn along with medium strong awns. Table IX shows these three groups of plants in relation to the three classes of awns.

TABLE IX.—*Relation of the hull character to the strength of the awn*

Hull character	Naked and intermediate, prevalence of naked.			Intermediate, hulled grain prevalent.			Hulled grain.		
Kind of awn	Weak.	Medium strong.	Strong.	Weak.	Medium strong.	Strong.	Weak.	Medium strong.	Strong.
Number	488	35	4	50	44	8	27	132	61
Percentage	*92.5*	*6.6*	*0.8*	*49.1*	*43.1*	*7.8*	*12*	*60*	*28*

The relation between the kind of awn and character of hull is very evident. In the naked and seminaked group practically all awns are weak. In the middle group approaching the firmly hulled type of grain, the weak awns on one hand and the medium and strong awns on the other occur in approximately equal numbers. In the third class a condition opposite to that of the first group is reached, where 88 per cent of the plants have medium strong and strong awns. It may be stated that the same tendency also prevails in regard to the quantitative distribution of the awns, the lowest number of awned spikelets being correlated with the weakest kind of awn and, conversely, plants with strong awns having usually all spikelets awned.

From the above discussion it is obvious that the quality and quantity of awns is limited in this cross by the morphological constitution of the lower flowering glume, which in turn is determined by the gene for nakedness. In studying the inheritance of the awns, just as in the case of the pubescence, it is necessary to disregard those plants with naked or almost naked grain. On these naked plants there is no opportunity for the somatic expression of strong awns, even if the gene for such awns is present.

In Table IX the two classes of plants included under the rubrics "Intermediate hulled grain prevalent" and "Hulled grain" may be classified accurately with reference to awn characters. On combining these two classes of plants there are in all 322 plants, of which 77 have weak awns and 245 have medium strong or strong awns. This is apparently a 1-to-3 ratio, the expected numbers being 80 to 241.5. However, again this result is based upon a selected group of F_2 plants, and it is possible that this group is not a random sample of the F_2 population with respect to awn characters.

SUMMARY

This paper contains a description of the F_1 and F_2 generations of a cross between a black hulled oat, *Avena sativa patula* var. Victor, and a white naked oat, *Avena sativa nuda* var. *inermis*.

The hulled parent is characterized by the presence of firm flowering glumes (paleæ) which adhere closely to the caryopsis, biflorous spikelets, black color of the glumes, strong awns, and a long but rather sparse pubescence at the sides of the base of the lower grain.

The naked parent is characterized by the presence of loose membranous flowering glumes which do not adhere to the caryopsis, multiflorous spikelets, white or light yellow glume color, almost total absence of awns and the absence of pubescence. It is possible that the absence of awns and of pubescence is due to the inability of these characters to express themselves on the thin membranous glumes.

The F_1 generation is distinctly intermediate in most characters. In regard to the glumes, both naked and firmly hulled grain as well as intermediate forms are found on the same panicle and even in the same spikelet. As shown in Table I, the spikelets near the top of the panicle are either entirely naked or nearly so, while those spikelets near the base of the panicle tend to be firmly hulled. A similar but less marked relation is to be observed between the spikelets at the tip and base of each whorl.

In the F_2 generation a large number of intermediate forms appear. In addition to the two parental hull types, four intermediate classes were distinguished. These intermediate forms contain all gradations from the plants with perfectly hulled grain to the perfectly naked forms.

As shown in Table II, the inheritance of the hull characters presents a simple Mendelian relation giving 1 hulled, 2 intermediate, 1 naked. Likewise, in respect to grain color, there are 3 black plants to 1 white in the second generation.

As shown in Tables III and IV, the genes for these two characters segregate independently of each other.

In all cases multiflorous spikelets occur only in connection with naked grain. Plants with completely hulled grain bear only biflorous spikelets.

The inheritance of the pubescence at the base of the lower grain presents some difficulties, since this character can not manifest itself on plants with naked grain. By using a selected group of plants having hulled and intermediate grain it is found that this pubescence behaves as a bifactorial character, giving 15 pubescent plants to 1 without pubescence (Table V). Neither of these genes are linked with the color genes.

The evidence available indicates that one of these pubescent genes may come from the naked parent.

The presence of long and short pubescence at the base of the grain behaves as a monohybrid character and segregates independently of the other genes considered.

A remarkable feature of this cross is the presence of pubescence at the base of the upper or second grain. There are no cultivated varieties of oats which possess this character. In the present cross these forms occur only on spikelets where the lower grain is naked or seminaked. It is probable that the presence of this pubescence at the base of the upper grain is due to physiological disturbances caused by the presence of the naked lower grain.

The presence of awns is also affected by the nature of the glumes. All naked grains bear only a thin, weak awn. On considering only the hulled and intermediate types of grain, there appears to be a simple 3-to-1 ratio between plants with medium strong to strong awns and those plants with weak awns.

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PLATE 39

A.—A head of the Victor oat, line 262. × 1/5.
B.—A head of the naked oat, *Avena sativa nuda* var. *inermis*. × 1/5.
C.—A head of an F_1 generation plant of the cross ♀ Victor × ♂ *Avena nuda*. × 1/5.

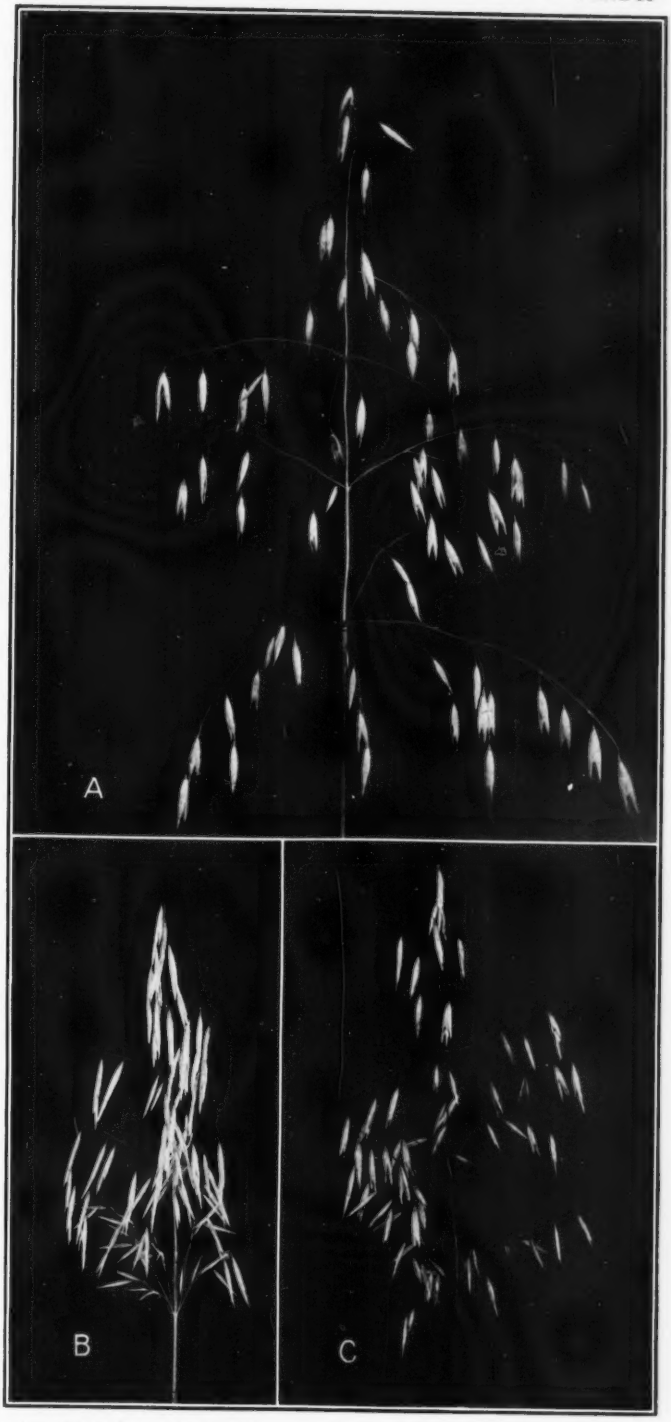

A
B
C

PLATE 40

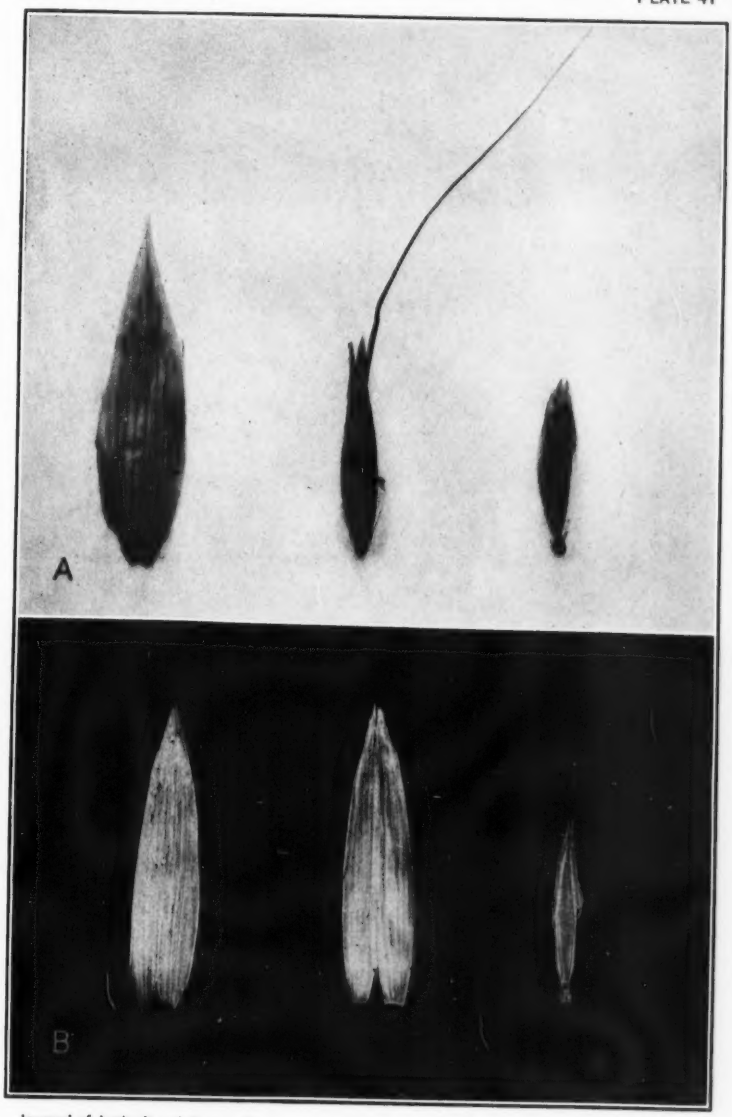
PLATE 40

A.—Single spikelet of *Avena nuda*. × 2.
B.—Single spikelet of the Victor oat. × 2.

PLATE 41

A.—Glumes of the Victor oat: From left to right—One of the outer, sterile, covering glumes, or the gluma; lower flowering glume, the palea inferior, and upper flowering glume, the palea superior. × 2.

B.—Glumes of the naked oat: From left to right—One of the glumæ, palea inferior, and palea superior. × 2.


A
B

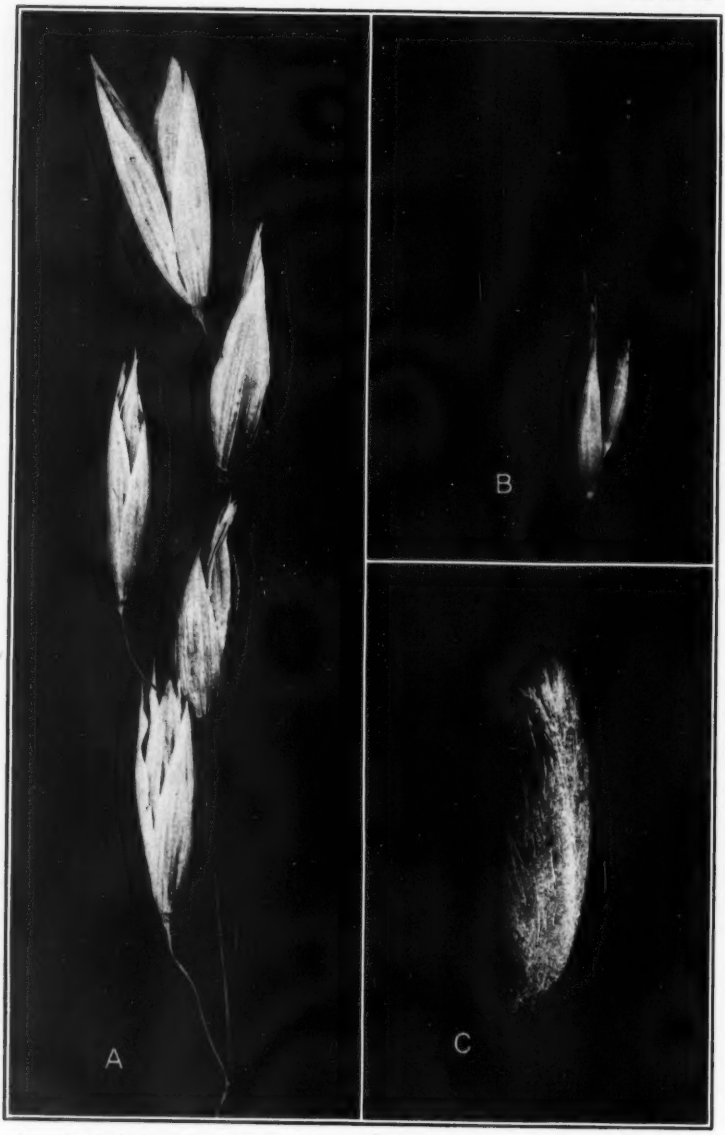

A
B
C

PLATE 42

A.—♀ Victor × ♂ *Avena nuda* F_2: A branch of a plant bearing biflorous spikelets containing naked kernels. × 2.

B.—♀ Victor × ♂ *Avena nuda* F_2: A white grain showing no pubescence at the base of the lower grain. × 2.

C.—Caryopsis of *Avena nuda*. × 5.

PLATE 43

A.—♀ Victor × ♂ *Avena nuda:* A head of an F_2 plant bearing only completely hulled grain. × 1/5.

B.—♀ Victor × ♂ *Avena nuda:* A head of an F_2 plant bearing only hull-less grain. × 1/5.

C.—♀ Victor × ♂ *Avena nuda* F_2: *a*, Spikelet with intermediately hulled grain; *b*, dorsal view of same. × 2.

A
B
C
a
b

PLATE 44

♀ Victor × ♂ *Avena nuda* F_1: Types of grain. From left to right—Multiflorous spikelet bearing naked grain, intermediately hulled grain, and completely hulled grain. × 2.

PLATE 45

♀ Victor × ♂ *Avena nuda* F_2: Types of grain segregating in the second generation. From left to right—Multiflorous spikelet bearing naked grain, spikelet bearing naked grain and showing reduction in number of florets, intermediately hulled grain and completely hulled grain. × 2.

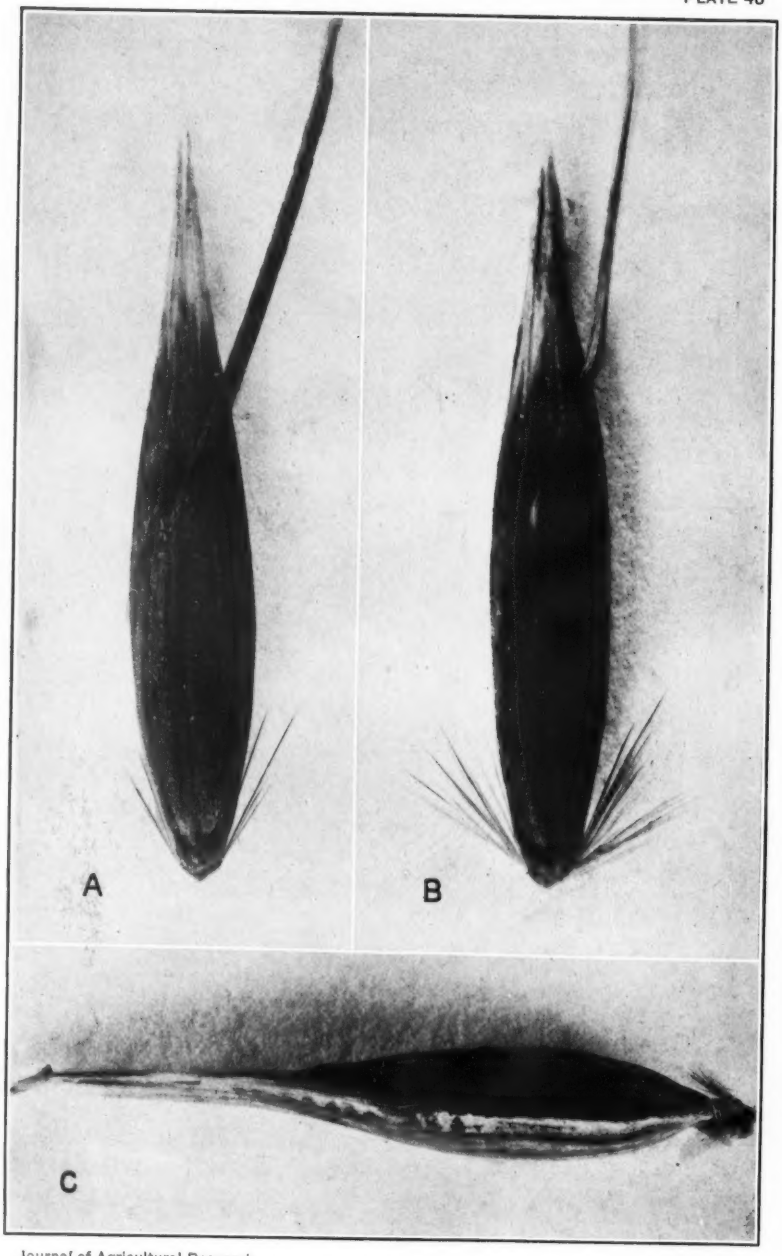

A
B
C

PLATE 46

A.—Lower grain of the Victor oat showing the long but sparse pubescence at the sides of the base. × 5.

B.—♀ Victor × ♂ *Avena nuda* F_2: Lower grain showing a rather thick pubescence at the sides of the base. × 5.

C.—♀ Victor × ♂ *Avena nuda* F_2: Dorsal view of upper grain showing pubescence at the sides of the base. × 5.

PLATE 47

♀ Victor × ♂ *Avena nuda* F_2: A spiklet showing pubescence on both lower and upper grain. ×5.

TWO NEW CAMBIUM MINERS (DIPTERA)

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HISTORICAL REVIEW

The two species of Agromyza discussed in this paper add two new cambium miners to science. The larvæ mine in the cambium of the living tree, the mine leaving a scar known as a "pith-ray fleck." [1] The mines are very much like those of *Agromyza pruinosa* Coq., the cambium miner in river birch (*Betula nigra*).[2] One of the species was reared from red maple (*Acer rubrum*) and the other from service berry, or shadbush (*Amelanchier canadensis*). Both of these species run to *A. setosa* in the table of Agromyza by Malloch.[3]

A dipterous cambium miner was first reared in Europe in 1906 by Nielsen,[4] and in 1913 the author reared an American species.[2] Later, in 1915, another species was reared by Grossenbacher [5] which was redescribed by Malloch the same year.[6]

CHARACTER OF TREES ATTACKED

The trees attacked are apparently healthy, and infested ones can not be detected by their outward appearance. The only way to detect the larva is to remove the bark and expose the cambium, where at a glance one can generally recognize the new galleries from the old ones, since the new larval mines are only faintly darker than the living cambium, whereas all the old work is generally brown.

CAMBIUM MINER IN RED MAPLE

DESCRIPTION

ADULT

Agromyza aceris, n. sp.

MALE AND FEMALE.—Black; frons opaque black, reddish along upper edge of lunule; width of frons about one-half that of head; orbits wide; four orbital bristles present, situated near inner edge of orbit; shiny around base of orbitals, a row of microscopic hairs between eye margin and orbitals; ocellar triangle slightly indicated

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Journal of Agricultural Research,
Washington, D. C.
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Vol. X, No. 6
Aug. 6, 1917
Key No. K—55

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THE CAMBIUM MINER IN SERVICE BERRY

DESCRIPTION

THE ADULT

Agromyza amelanchieris, n. sp.[1]

MALE AND FEMALE.—This species closely resembles *Agromyza aceris*, but is easily separated from it by its smaller size and the following differences: Black, slightly more opaque on the frons and thorax. Frons faintly depressed in the center, half as wide as head; red area above lunule wide; orbits fairly wide; five orbital bristles; microscopic hair present; ocellar triangle with longer black hairs; area between lunule and base of antennæ narrower and paler yellow; first and second joints of antennæ dull, dark reddish brown; third joint rounded, dull black, inner basal corner reddish; arista black, only slightly longer than upper orbital bristles; face opaque black, keel indistinct; facial depression with a very faint reddish reflection on each side; narrow red area on each side of face wanting; along upper part of oral margin narrower, reddish yellow; dorsum of thorax nearly opaque; abdomen blacker and more shining than thorax; hypopygium of male entirely black; legs, knees, and trochanters black; wings hyalin, veins nearly black; outer cross vein its own length from inner cross vein.

Length, male and female, 3 to 3.5 mm.

TYPE LOCALITY.—French Creek, W. Va. Mr. F. E. Brooks, collector.

TYPE.—Female, Cat. No. 21063, United States National Museum.

ALLOTYPE.—Male.

Described from seven specimens.

THE LARVA

The larva (Pl. 48, *C*) is quite similar to that of *Agromyza aceris*, except for the following differences: It is longer and more slender; the cephalopharyngeal skeleton is longer and not so robust; the anterior and posterior pairs of spiracles are slightly smaller; and the small chitinous plates on the sides, below the middle of the last segment, are arranged in four rows instead of three.

For further details see Plate 48, *C*.

Length, 20 to 25 mm.; diameter, 0.65 to 0.85 mm.

THE PUPA

The pupa (Pl. 48, *D*) is paler yellow and very much more pointed than that of *Agromyza aceris*. The grooves along the segmental lines are not so pronounced. The cephalic end of the pupal case is missing. The posterior spiracles are reddish, not quite so prominent and a little more elongated on the apex, plainly showing the three circular plates with the dorsal slit. The anal depression is reddish in the center. Otherwise it is like *A. aceris*, although very slightly smaller.

SEASONAL HISTORY

During June and the early part of July the larvæ were collected at French Creek. W. Va. They were nearly full grown and were taken from the trunk near the ground and from the roots. According to Mr. F. E. Brooks, who discovered them, "the larvæ leave a threadlike reddish line in the cambium." Nearly full-grown larvæ were collected at French Creek from June 10 to July 6, 1915. The time of pupation

[1] Submitted to the writer for study through the courtesy of Dr. A. L. Quaintance, Entomologist in Charge of Deciduous Fruit Insect Investigations, Bureau of Entomology.

stem is black, cylindrical, and the end is very faintly dented where it articulates with the anterior portion. From the posterior end of this stem are two long, flat, chitinous blades which are much longer than broad. They are dark brown above and black on the lower edge where they are slightly more chitinized. The muscles appear to be attached to the inside surface of these blades. On the underside, where the two large blades branch out from the stem, is a fingerlike projection, pointing backward. This projection is hollow and U-shaped and is divided at the apex. It is brownish above and black on the underside. For further details see Plate 48, *A*, *a*.

Along the upper front edge of the first segment next to the head there are small, impressed, parallel lines and the lower portion is covered with numerous, very short, yellowish brown spines. On the dorsum of this segment, just in front of the middle, are located the anterior spiracles; they are T-shaped, yellow and very faintly raised above the surface. From a dorsal view they have about 8 or 10 microscopic ringlets around the edge, giving the edges a scalloped appearance. Back of these spiracles is a faint depression formed by a transverse fold or wrinkle. The segmental lines are rather weak and the segmentation can be plainly seen on the plate. Along the basal segmental line of the last segment are three rows of microscopic, yellow, chitinous plates; the first row (toward the head) is broken on the dorsum only, the middle one is broken on the dorsum and venter while the third row is just a short row on each side. Just below the middle of the last segment are three short rows of the same plates on each side of the larva. The posterior spiracles are situated on two tubercles located on the dorsal, apical portion of the last segment. These tubercles are pale yellow and flattened along the apex. On this apical edge are three brown, nearly round, chitinous plates, each having a transverse dorsal slit. For details see Plate 48, *A*, *b*. On the ventral side near the apex is a large tubercle with the anal opening located on the apex.

Average length, 15 to 17 mm.; average diameter, 0.75 to 1 mm.

PUPA

The pupa (Pl. 48, *B*) is pale yellow, cylindrical, and tapers very slightly toward each end. It is faintly shiny and is formed by the shrinkage of the larva. The head is entirely retracted, leaving 11 segments plainly visible. All segments are marked with well-defined grooves. The entire surface of the pupal case is faintly marked with transverse striæ. All the segments are nearly uniform in width except those on each end, which are narrower. The place where the head was retracted is marked by a small puckered surface, faintly reddish. The anterior and posterior pairs of spiracles are formed by the shrinkage of those of the larva into small reddish brown, chitinous tubercles. The anal area is marked by a circular depression which is nearly black in the center. The adult emerges through a slit made along the lateral edge of the first three or four segments.

Length of male, 4 mm.; diameter, 1.65 mm. Length of female, 5 mm.; diameter, 2 mm.

SEASONAL HISTORY

During July and August the larvæ were fairly common at Falls Church, Va., and the surrounding country. They mine down the cambium in the trunk and roots of *Acer rubrum*, and, after reaching maturity, make their exit, pupating in the ground about ½ to 1 inch to the side of the exit hole. Full-grown larvæ were collected from July 10 to August 19 by Mr. T. E. Snyder and the writer. Full-grown larvæ were also taken at French Creek, West Virginia, on July 11, 1916, by Mr. F. E. Brooks.

Pupation took place from July 10 until the latter part of August, and the species remained in the pupa stage during the winter.

Adults emerged from April 24 to 26, 1916.

and covered with numerous short black hairs; ocelli pale yellow; ocellar bristles large and directed obliquely forward; below lunule to base of antennæ dull clay yellow; first joint of antennæ clay-yellow, second and third joints dull reddish brown, second joint with a light reflection on upper edge and the third joint rounded, only slightly wider than long, blackish on upper edge; arista nearly black, thickened at base, brownish above thickened portion; pubescence of antennæ indistinct; arista nearly twice as long as upper orbital bristle; center of face opaque black, keel indistinct, a narrow area on each side of face, extending from base of antennæ to cheeks, dull dark red; opaque black of frons extending down around eye in a narrow area; across lower part of face, along oral margin, an arcuate, dull clay-yellow band with the pointed ends extending down along the oral margin (narrower in female); ridge along sides of oral margin black; vibrissæ distinct; cheeks dull black; proboscis and palpi black, palpi with several bristles; dorsum of thorax black, subshining, with a faint grayish appearance and thickly covered with bristly hairs; four dorsocentrals; the pair of bristles between last pair of dorsocentrals about three-fourths as large as the latter; pleura concolorous; scutellum bare, concolorous, with two pairs of marginal macrochætæ, apical pair decussate; stem of halteres yellowish brown, knobs nearly white; abdomen concolorous, covered with numerous hairs; marginal bristles distinct; hypopygium of male dull yellowish brown, darker toward apex; legs black, knees and trochanters yellowish; middle tibiæ bearing several distinct bristles on posterior side near middle; wings hyalin, veins yellowish brown, costal vein and apex of first vein dark brown; costal vein reaching fourth vein; first costal division about three-fourths as long as second; inner cross vein slightly before apex of first vein; outer cross vein slightly more than its own length from the inner crossvein; last section of fifth vein distinctly longer than penultimate section; veins 3 and 4 slightly divergent at apices.

Reared by the writer at the Eastern Field Station of the Forest Insect Investigations, Bureau of Entomology, at Falls Church, Virginia.

Length, male, 4 mm.; female, 4.5 mm.

TYPE LOCALITY.—Falls Church, Va.

TYPE.—Female. Catalogue No. 21062, United States National Museum.

ALLOTYPE.—Male.

The male of this species has two distinctly abnormal bristles on the head, one in front of the postvertical pair and the other in the right fronto-orbital row.

LARVA

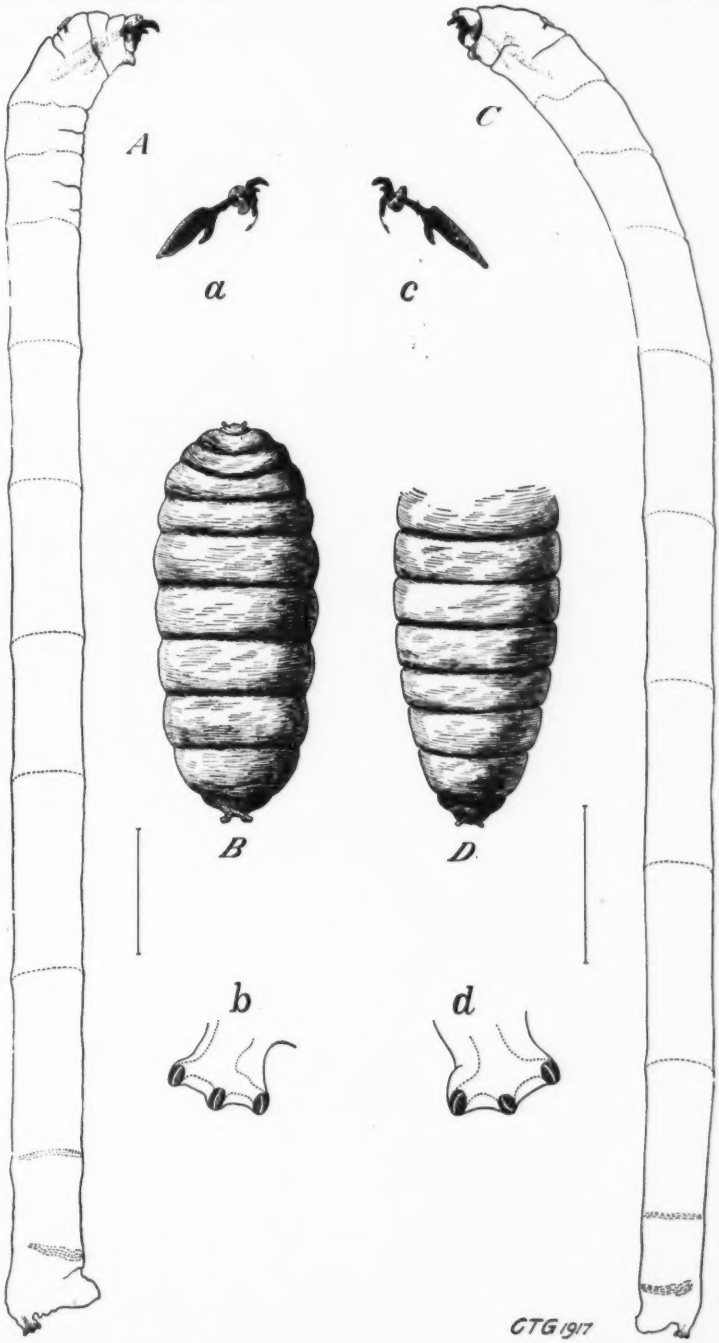
The larva (Pl. 48, *A*) is opaque white, cylindrical, and tapering very slightly at extreme anterior and posterior ends. Head segment small and not retractile. Above the hooklet and attached to it is a small, fleshy, conical portion. On each side of this conical portion, near the apex, is a small, black, chitinous, transverse plate with two small, black, cylindrical, chitinous pieces between and perpendicular to these two transverse plates. The hooklet complete (cephalopharyngeal skeleton) is shown in Plate 48, *A*, *a*. It is divided into two parts. The apical portion is black and highly chitinized and the greater part of it is exposed. The upper portion extends forward in the form of a large, robust, clawlike tooth; below this are two smaller teeth extending obliquely forward. The tooth on the right side of the larva is smaller than that on the left. The bottom terminates in a sharp toothlike point above which are attached two narrow, brownish, chitinous bands extending slightly below the bottom point. Just above the lower end of, and attached to the band, is a small, blacker chitinous piece. These bands appear to afford muscle attachment for operating the hooklet. The back terminates in a short, stout, oblique point where it articulates with the basal portion. On each side and in front of this rear point is attached a brownish black chitinous plate which is nearly quadrate and is attached along its anterior edge. This plate resembles an eye in the larva. The basal portion of the hooklet has a rather peculiar construction and is quite elongate and flattened. The

is not known. Mr. Brooks states that flies issued at French Creek from April 13 to 17, 1916, and that adults were collected from branches and buds at the same locality on April 18, 1916.

Nearly full-grown larvæ were collected at Smoky Mountain Crest, on the boundary line between Tennessee and North Carolina, on July 14, 1913, by Mr. T. E. Snyder.

PLATE 48

A.—*Agromyza aceris:* Larva. *a*, Cephalopharyngeal skeleton; *b*, posterior spiracle.
B.—*Agromyza aceris:* Pupa.
C.—*Agromyza amelanchieris:* Larva. *c*, Cephalopharyngeal skeleton; *d*, posterior spiracle.
D.—*Agromyza amelanchieris:* Pupa.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